Study of Hydrogel based Controlled Release Drug Delivery System for Captopril and its *in-vitro*, *in-vivo* Evaluation

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DOCTOR OF PHILOSOPHY

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by

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In the name of Allah, the Most Merciful, the Most Kind

DEDICATION

Dedicated to my beloved parents, wife and children

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Foremost, I should bow to **ALLAH ALMIGHTY** who made me able to work and accomplish my work within time .All respects are for The Last Prophet, **HAZRAT MUHAMMAD** (Peace Be Upon Him),who enable us to recognize our Creator.

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Chapter no.1

Introduction

INTRODUCTION

A remarkable advancement has been made in dosage form design; more progress is yet to be made for treating a number of clinical diseases. The drug administration should be in a manner that its concentration achieved matches the physiological needs at predefined periods of time and at proper site for desired therapeutic action. An optimum concentration of drug will overcome the side effects related to conventional dosage forms. This can ultimately lead to cost effective treatment by minimizing the overall expenses. Evolution of an existing drug molecule from a conventional form to a novel delivery system can significantly improve its performance in terms of patient compliance, safety, and efficacy. These days, drug manufacturing companies are engaged in the development of multiple platform technologies to get competitive advantage, extend patent life, and increase market share of their products.

Various controlled release drug delivery systems have been formulated and are under progress, e.g. matrix systems, hydrogels, microcapsules, microspheres, liposomes, nanoparticles and many more. Developing new drug delivery technologies and utilizing them in product development is critical for pharmaceutical companies to survive. Advances in drug delivery are occurring at a rapid pace, and it is important to keep up with innovations and applications of these technologies. Considerable progress has been made in hydrogels syntheses and applications, that is playing a key role in controlled drug delivery technology. The polymers coming from natural, renewable sources, nontoxicity and biocompatibility, hydrogels also have economic advantages over other drug delivery systems. They are easy

and economical to synthesize requiring simple preparation methods to crosslink the polymers. The Hydrogels have been extensively used in the development of smart drug delivery systems. Synthesis of new polymers, polymer combinations in different ratios, crosslinking agents with more biocompatibility and better biodegradability would be essential for successful applications.

With ever-growing advancement of research in the field of pharmaceutical technology, hydrogels have received considerable attention as convenient, biocompatible and stable carrier for a wide range of drugs, such as NSAIDs (non-steroidal anti-inflammatory drugs), antihypertensives, pharmaceutical proteins and peptides. Hydrogels protect the degradation of drugs from unfavorable conditions and control the drug release by changing the gel structure in response to environmental stimuli such as temperature, pH, ionic strength, solute concentration, electric field, magnetic field, light, sound etc. This ensures acceptable drug stability conforming official standards. Hydrogels are known to reduce the problems of both conventional and novel drug delivery systems.[1,2] They are extensively used in the area of pharmaceutical and medical applications such as for controlled drug release and delivery, tissue engineering and regenerative medicine. They have been designed for drug targeting by using biocompatible polymers along with drug in micronized form and attaching "homing devices" like antibodies. It protects the normal cells and targets the diseased ones.[3,4]

Hydrogels are generally defined as two- or multicomponent systems consisting of a three-dimensional network of hydrophilic polymers bound by crosslinking or other cohesive forces, and can absorb large quantities of water while maintaining the structure. The crosslinking of the hydrophilic polymer chains prevent their dissolution. Depending on the properties of the polymer(s) used, as well as on the nature and density of the network joints, such structures in equilibrium can swell and retain a significant portion of water when placed in an aqueous solution. In the swollen state, the mass fraction of water in a hydrogel is much higher than the mass fraction of polymer. Their affinity to absorb water is due to the presence of hydrophilic groups such as –OH, -CONH, -$CONH_2$, -SO_3H etc. in the polymers forming hydrogel structures.[5-7] Depending upon the nature of aqueous environment and polymer composition, the polymer can be hydrated up to more than 90% due to the contribution of these groups and domains in the polymer's network.[8] Hydrogels can be

formed by physical or chemical crosslinking of homopolymers or copolymers. Two general classes of hydrogels can be defined - physical gels (pseudogels), as well as chemical gels (true, permanent). In physical hydrogels, the networks are connected by non-covalent interactions, such as electrostatic forces, hydrogen bonds, protein interactions, hydrophobic interactions or chain entanglements (such gels are non-permanent and usually they can be converted to polymer solutions by stress/heating). The other is chemical hydrogels with covalent bonds (replacing hydrogen bond by a stronger and stable covalent bonds) linking the chains. Chemical crosslinking methods include radical polymerization, chemical reactions of functional groups, high-energy radiations and enzyme usage. They attain an equilibrium swelling state which is dependent upon interaction polymer with water of and the crosslink density.[9-11]

A broad range of synthetic and also natural polymers have been used in the synthesis of hydrogels. Usually the materials applied for general-purpose hydrogels are poly (ethylene oxide), poly(vinyl alcohol), polyvinylpyrrolidone, poly(hydroxyethyl methacrylate) and cellulose derivatives such as Hydroxypropyl methylcellulose (HPMC), Methylcellulose (MC), Carboxymethylcellulose (CMC) etc. Owing to this, a new class of hydrogels known as environment sensitive hydrogels, capable of reacting to various physical and chemical stimuli such as temperature, pH, ionic strength, solute concentration, electric field, magnetic field, light, sound etc., have been tested for use in the so-called "intelligent biomaterials".[12-15]

In Pharmaceutical technology, the research study has objectives related to betterment of the health care system to improve the quality of life. This research project is concerned with the formulation and characterization of hydrogels. Hydrogels are known to reduce the problems of both conventional and novel drug delivery systems. They are extensively used in the area of pharmaceutical and medical applications such as for controlled drug release and delivery, tissue engineering and regenerative medicine.

This work was aimed to develop an orally administered controlled release hydrogel formation loading an antihypertensive drug “Captopril”. Different polymers, monomers were used in various combinations and subjected to *In-vitro* and *In-vivo* characterizations. Oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance, cost

effective manufacturing process and flexibility in formulation. Extensive studies are required to examine the factors that play role in development of controlled release formulations. Despite of the already existing research work, there are likely to be no well-established captopril controlled release formulations reported in the market. Development of a once daily captopril oral formulation would be a significant advantage for patient compliance accompanied by minimization of the drug side effects as a result of reduction in the drug blood concentration fluctuations, especially in long-term therapy. The hydrogel based dosage system that provides sustained release without the need to use special coatings or structures, both of which also add to the cost of manufacturing. Hence, a cost effective treatment will be provided in hypertension management.

Chapter no.2

Literature Review

Hydrogels are crosslinked polymeric networks with the ability to swell in an aqueous medium. Crosslinking in hydrogels occurs by chemical or physical means depending on the properties of various polymers, monomers, crosslinking agents and experimental conditions adopted for their synthesis. Due to different types of chemical structure and variety of crosslinking methods, a wide range of hydrogels have been prepared for various applications in pharmaceutical and biomedical fields. This chapter describes hydrogel classification, their methods of preparation, characterizations and applications.

The swelling behavior of hydrogel formulations depends upon various factors, such as the nature of the polymer, the polymer-solvent compatibility and the degree of crosslinking. The polymeric network becomes more hydrophilic as the degree of ionization increases and the drug loading as well as release is dependent upon the swellability of the polymer. Depending upon the polymer's structure, the hydrogels can undergo significant volume changes in response to slight changes in environment which can involve pH, temperature, the composition of the surrounding liquid etc. The hydrogels are usually classified according to their response to their response to environmental stimuli as given below:

2.1 Types of hydrogels

2.1.1 pH sensitive or ionic hydrogels

The ionic hydrogels respond to changes in pH of the external environment. They can be anionic or cationic, due to the presence of certain ionic groups. Some of the pH sensitive polymers used in hydrogels' preparations are polymethyl methacrylate (PMMA), polyacrylamide (PAAm), methacrylic acid (MAA), polyacrylic acid (PAA), polyethylene glycol and poly dimethlaminoethylmethacrylate (PDEAEMA). Acrylic acid (AA) and methacrylic acid (MAA) are the most commonly used monomer to fabricate anionic hydrogels.[16-18] The copolymer of bacterial cellulose and acrylic acid, which are anionic copolymers, swell high in neutral or high pH but do not swell in acidic medium.[19] On the other hand, poly-dimethyl-amino-ethylmethacrylate (PDEAEMA)[20] and some cellulose derivatives have been used in cationic hydrogel formation. Two cationic hydroxyethylcelluloses of different hydroxyethyl and ammonium group contents were crosslinked and loaded with diclofenac sodium, with which they interacted through ionic and hydrophobic bonding at acidic pH. As the pH is increased up to 8 the interactions break and release process was sustained for more than four hours.[21]

The pH- sensitive hydrogels have mainly been used to encapsulate proteins and peptides for oral administration. Other drugs have been delivered such as ketoprofen, caffeine, diclofenac sodium and anticancer drugs. The composite hydrogel, based on a methacrylated and succinic derivatives due to its pH-sensitive swelling and enzymatic degradability, together with mucoadhesion and cell compatibility, could be potentially useful as system for the oral treatment of colonic cancer, choosing 2-methoxyestradiol as a model of anticancer drug.[22]

2.1.2 Temperature sensitive hydrogels

These environment sensitive hydrogels have ability to swell or/and deswell as a result of changes in temperature. Thermoresponsive hydrogels have led to dramatic advances in the bioengineering and biotechnological fields.[23,24] They have gained considerable attention for delivering large number of temperature sensitive drugs. The release and mechanical characteristics of both drug and hydrogels are altered with the change in the temperature of external environment.[25] The hydrogel of polymers bearing N-isopropylacrylamide (NIPAAm) and acrylamide (AAm) so synthesized showed variable physical appearance from transparent solution to translucent gel depending upon temperature and was utilized to entrap insulin for prolonged release.[26,27] Another thermo-sensitive hydrogel comprising of

polyorganophosphazene with amino- omegamethylpolyethylene glycol was formulated for delivering human growth hormone.[28, 29]

Many polymers exhibit a temperature-responsive phase transition property. The common characteristics of these hydrogels are the presence of hydrophobic groups such as methyl, ethyl and propyl groups. Most commonly used are poly N-isopropylacrylamide (PNIPAAm), Poly (N, N-diethylacrylamide (PDEAAm), copolymers of NIPAAm can also be made using other monomers, e.g. butyl methacrylate (BMA), to alter the lower critical solution temperature (LCST). They can be sub-categorized into negatively thermosensitive and positively thermosensitive gels. Negative thermo-sensitive hydrogels contract upon heating above their low critical solution temperature. The poly N-isopropylacrylamide (PNIPAAm) hydrogel is well-known thermosensitive hydrogels for biomedical applications, because of its lower critical solution temperature (LCST) at around 32 ºC in aqueous solution. When solution temperature is below LCST, the network expands; it is extremely soluble in water and appears transparent. PNIPAAm chains contract and dehydrated when heated to a temperature above its LCST. At this point, PNIPAAm precipitates out from the aqueous solution, appearing opaque.[30]

Certain hydrogels swell at high temperature and shrink at low temperature are termed as positive thermosensitivity. For example, inter penetrating polymer networks (IPNs) of poly (acrylic acid) and polyacrylamide (PAAm) or Poly(AAm–co-BMA) exhibits positive temperature dependence of swelling.[31]

2.1.3 Glucose sensitive hydrogels

Glucose-responsive hydrogels, exhibiting response to glucose concentration, are widely applicable in biosensing, microfluidics and bio-microelectromechanical systems, as well as implantable drug delivery systems for diabetes management applications.[32-34] Four types of glucose-sensitive hydrogels have been intensively investigated, which are on the basis of glucose oxidase,[35] concanavalin A,[36] phenylboronic acid[37] and glucose binding protein.[38]

The development of modulated insulin delivery systems is one of the challenging problems in controlled drug delivery area, as insulin has to be delivered in exact amount and time. Due to outstanding mechanical swelling properties, the glucose-sensitive hydrogels are promising

biomaterials for development of smart insulin delivery systems. As the glucose concentration increases, the crosslinking density of the gel decreases and the gel swells or erodes to release the insulin.[39, 40]

These hydrogels are usually based on glucose biosensor, which is sensitized to glucose concentration. A series of glucose-sensitive hydrogels based on glycidyl methacrylate modified dextran (Dex-G), ethylene glycol acrylate methacrylate modified concanavalin A (Con A–E) and poly (ethylene glycol) dimethacrylate (PEGDMA) were synthesized by photopolymerization. The hydrogels were highly glucose sensitive and biocompatibile, which could be prospectively applied as glucose biosensor and intelligent insulin delivery carrier.[41]

Glucose-sensitive hydrogels (GSHs) responsive to both pH value and glucose concentration have also been prepared by polymerizing solutions containing hydroxypropyl methacrylate, (N, N-dimethylamino) ethyl methacrylate, and tetraethylene glycol dimethacrylate in the mole ratio 70:30:2.[42]

2.1.4 Other stimuli sensitive hydrogels

Temperature, pH and glucose sensitive hydrogels, have gained considerable attention in the field of drug delivery. However, other stimuli like light, electric field, pressure, protein sensitive hydrogels have been utilized in formulation of responsive hydrogels, but these have limited applications in this area.[43,44]

2.1.4.1 *Electro-sensitive hydrogels*

Electric current is another envoirnmental signal to induce responses in hydrogel. These electro-sensitive hydrogels are usually synthesized from polyelectrolytes, which undergo shrinking or swelling in the presence of an applied electric field. Various conditions affect the swelling, shrinkage and bending of hydrogels. The hydrogels may show variation in responses when placed in water (or acetone- water mixture) in contact with electrode to that without touching the electrode. The presence or absence of electrolytes in the aqueous solution can also influence the results.

Application of electric field causes the shrinkage of hydrogels, which recover their original size as the electric field is turned off. This property of has been used for the modulated drug delivery by 'on–off' of the electric field.[45] Poly (2-acrylamido-2-methylpropane sulfonic acid– co-n-butylmethacrylate). Hydrogels have ablity to release edrophonium chloride and hydrocortisone in a pulsatile manner using electric current.[46]

2.1.4.2 *Light-sensitive hydrogels*

Light-sensitive hydrogels have potential applications in developing optical switches, display units and ophthalmic drug delivery devices. Light-sensitive hydrogels can either be UV-sensitive or visible light-sensitive hydrogels.[47]

The UV-sensitive hydrogels were synthesized by introducing a leuco derivative molecule, bis(4-dimethylamino) phenylmethyl leucocyanide, into the polymeric network. The UV light-induced swelling was due to an increase in osmotic pressure within the gel due to the appearance of cyanide ions formed by UV irradiations.

Visible light-sensitive hydrogels were prepared by introducing a light-sensitive chromophore (e.g. trisodium salt of copper chlorophyllin) to poly (N-isopropylacrylamide) hydrogels.[48] The Visible light exposure (e.g. 488 nm), of Hydrogels causes light absorption in chromophore, where it is dissipated as heat and raises the local temperature. It alters the swelling behavior of poly (N-iso propylacrylamide) hydrogels, which are thermo sensitive hydrogels.

2.1.4.3 *Pressure-sensitive hydrogels*

The pressure sensitivity appeared to be a common characteristic of temperature-sensitive gels. It was concluded that the pressure sensitivity of the temperature-sensitive gels was due to an increase in their LCST value with pressure.[49]

The degree of swelling of poly (N-isopropylacrylamide) hydrogels increased under hydro static pressure when the temperature is close to its LCST. Other hydrogels, such as poly (N-n-propylacrylamide), poly (N, N-diethylacrylamide) and poly (N-isopropylacrylamide), all showed the pressure sensitivity near their LCSTs.[50]

2.1.4.4 *Protein-sensitive hydrogels*

Stimuli-sensitive hydrogels can sense environmental changes and induce structural changes by themselves. They have attracted considerable attention as intelligent materials in the biochemical and biomedical fields. In particular, biomolecule sensitive hydrogels that respond to specific biomolecules have become increasingly important because of their potential applications in the development of biomaterials and drug delivery systems. The protein-sensitive hydrogels including enzymatically degradable hydrogels and antigen sensitive hydrogels undergo swelling changes in response to larger biomolecules.[51]

Biodegradable polymers have high potential in biomedical fields because of their increasing importance in genetic engineering and drug delivery systems. They can be digested by specific enzymes, for this they are used in formulation of enzyme sensitive hydrogels. Hovgaard *et al.*[52] focused on the fact that microbial enzymes in the colon, such as dextranases, can degrade the polysaccharide dextran. They prepared dextran hydrogels cross-linked with diisocyanate for colon specific drug delivery.

An antibody has recognition sites to bind with a specific antigen through multiple noncovalent bonds such as electrostatic interactions, hydrogen bonds, hydrophobic interactions, and van der Waals interactions. Antigen-sensitive hydrogels were prepared by using antigen–antibody bonds at cross-linking points in the hydrogels.[53, 54]

To investigate the possibility of an antigen-sensitive hydrogel as an intelligent system for novel drug delivery applications, the permeation of a model drug through an antigen–antibody semi-interpenetrating polymer network (semi-IPN) hydrogel membrane was investigated in the presence and absence of rabbit IgG as a free antigen.[54]

2.1.4.5 Microgels and nanogels

Apart from the synthesis of macroscopic networks, the hydrogels can be confined into smaller dimensions such as microgels. When the microgel particles are submicronized, they are known as nanogels. They have unique advantage of tunable size from nanometers to micrometers.[55,56] They possess high water content, biocompatibility and adjustable mechanical properties. The properties provide a unique mode for targeted delivery of

encapsulated drugs via blood circulation. Nanocarriers due to their size smaller than typical blood cells can be administered intravenously. They can freely float in the bloodstream into the smallest vessels/capillaries and achieve the target site- or tissue-specific delivery.[57, 58]

There are recent developments of microgel or nanogel particles as drug delivery carriers for biological, biomedical and drug delivery applications. They have also received attention as environmentally responsive systems and now are widely used as carriers for therapeutic drugs and diagnostic agents. They release the entrapped drug by swelling caused by change in the pH of the surrounding environment. For example, an anticancer drug adriamycin delivered to tumor cells showed the highest release at pH below 6.8.[59]

2.2 Methods of hydrogel preparation

As discussed earlier, both chemical and physical methods have been used by scientists to develop chemical and physical hydrogels, respectively. The widely used novel crosslinking methods to create the hydrogels are mentioned in figure 1 and they will be briefly discussed.

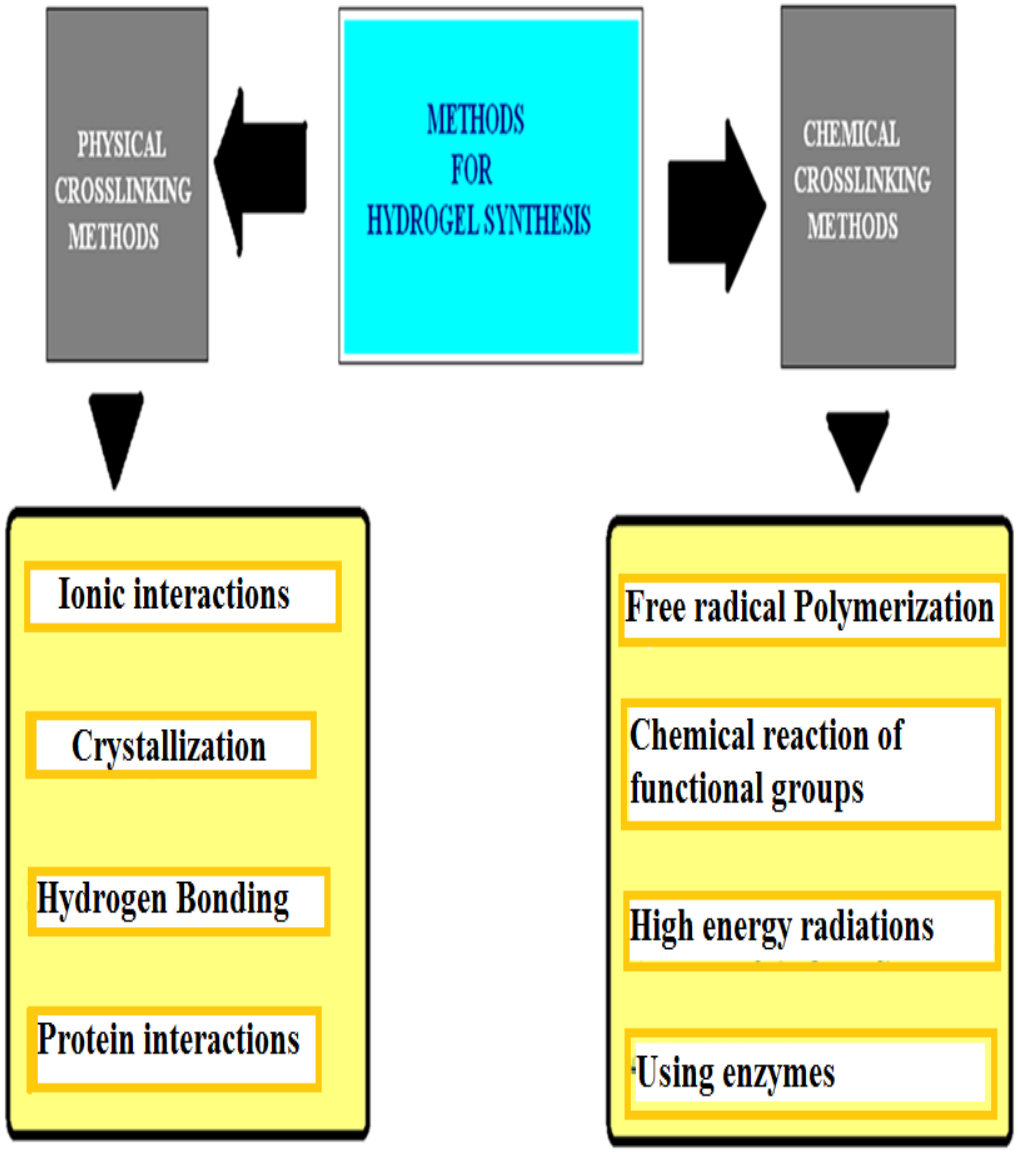

Figure 1. Methods for hydrogel preparation

2.2.1 Chemical Crosslinking methods

2.2.1.1 *Crosslinking by radical polymerization*

During polymerization process the monomer molecules are crosslinked chemically, resulting in the formation of either linear chains or a three-dimensional network of polymer chains.

Radical polymerization is one of the commonly used methods to synthesize the hydrogels, where low molecular weight monomers are crosslinked in the presence of crosslinking agents. A variety of hydrogels can be designed by this procedure, for example, different stimuli sensitive materials, hydrogels using water-soluble (synthetic, semi-synthetic and natural) polymers.[11]

In an attempt to determine the optimum conditions for hydrogel synthesis by the free-radical polymerization of sorbitan methacrylate (SMA), the hydrogel used in this study was well polymerized under the following conditions: 50% (w/v) SMA as monomer, 1% (w/w), α, α'-azo-bis (isobutyro-nitrile) as thermal initiator, and 1% (w/w) ethylene glycol dimethacrylate as cross-linking agent. Under these conditions, the moisture content of the polymerized SMA hydrogel was higher than in the other conditions and also from poly (methyl methacrylate [MMA]) hydrogels.[60]

Triblock copolymers prepared via consecutive atom transfer radical polymerizations using monomers, N-isopropylacrylamide (NIPAAm), (2-dimethyl amino) ethyl methacrylate (DMAEMA) and 2-hydroxyethyl methacrylate (HEMA), in the presence of ethylene glycol di-2-bromoisobutyrate as initiator. The so-prepared hydrogels exhibited both temperature- and pH-sensitive behavior.[61]

2.2.1.2 *Crosslinking by chemical reaction of functional groups*

The water solubility of the polymers is attributed to the presence of functional groups like -OH, -COOH, -NH_2, used to create hydrogels. The hydrogels are formed by the covalent bonding between the polymer chains and functional groups, such as amine-carboxylic acid, isocyanate-OH/NH_2 or by Schiff base formation. Water-soluble polymers with hydroxyl groups can be crosslinked with aldehydes e.g. crosslinking of poly (vinyl alcohol) can be crosslinked using glutaraldehyde.

Crosslinking of water soluble polymers can also occur by the addition reactions, where the hydrogels are formed using higher functional cross linking agents such as 1,6-hexamethylene-diisocyanate, di-vinyl sulfone and many other reagents react with functional groups of water-soluble polymers. Other frequently applied synthesis of hydrogels involves condensation reactions between hydroxyl groups or amines with carboxylic acids or

derivatives to yield polyesters and polyamides, respectively. N, N-(3-dimethylaminopropyl)-N-ethyl carbodiimide (EDC) is an efficient reagent to establish chemical crosslinking of water-soluble polymers with amide bonds in the preparation of gelatin hydrogels.[62, 63]

2.2.1.3 *Crosslinking by high-energy irradiation*

Nevertheless, owing to unique advantages such as shorter reaction times, crosslinking under mild conditions (room temperature and physiological pH), higher yields; limited generation of by-products and relatively easy scale-up without detrimental effects, radiation-assisted preparation of hydrogels have become an appealing synthetic tool. In addition, simultaneous synthesis and sterilization of hydrogels are the unique advantages of radiation processing.[64-69]

The permeability and swelling characteristics of the formed gel are dependent upon the amount of polymer and radiation intensity. Usually the crosslink density increases with increasing polymer concentration and radiation dose.[70]

Particularly gamma radiations, electron beams as well as microwave radiations are used for polymerization. Gamma radiation was used to crosslink the biodegradable hydrogels based on an acryloylated poly-aspartamide.[71] An environment sensitive bacterial cellulose and acrylic acid composite was formed via electron beam. It exhibited higher swelling ability and the degree of swelling increased as the pH of surrounding medium increased.[19] Similarly temperature-sensitive poly (N-isopropylacrylamide) PNIPAAm hydrogels were prepared by microwave irradiation using Mars-5 microwave accelerator.[72] However the domestic microwave ovens are more convenient source of microwave radiation to create the chemical crosslinking among a variety of monomers and polymers. A copolymer hydrogel of k-carrageenan (kC) and acrylamide (AAm), has been synthesized in aqueous medium at pH 7 in the presence of the initiator potassium persulfate (KPS), by microwave irradiation using LG make domestic microwave oven.[73]

2.2.1.4 *Crosslinking using enzymes*

An emerging and interesting approach for formation of hydrogels is based on enzyme-catalyzed crosslinking reactions. The enzymes from various sources, such as microbial Transglutaminase and mushroom tyrosinase provide a method for creating gels and may offer

interesting opportunities for in situ applications. The ability of these two enzymes to catalyze the formation of gels from solutions of gelatin and chitosan was observed and compared.[74]

Similarly, hydrogels were synthesized from glutaminamide-functionalized poly (ethylene glycol) (PEG) and poly (lysine-co-phenylalanine) using transglutaminase (TG) in the presence of calcium ions as cofactors. The covalent crosslinking occurred by formation of an amide linkage between the carboxamide groups of peptidyl glutamine residues and primary amine groups of lysine residues.[75]

Enzyme-mediated redox chain initiation involving glucose oxidase (GOX) was employed in a dip-coating technique to polymerize multiple, three-dimensional hydrogel layers using mild aqueous conditions at ambient temperature and oxygen levels.[76]

Dextran hydrogels were formed in situ by enzymatic crosslinking of dextran-tyramine conjugates and their mechanical; swelling and degradation properties were evaluated. These results demonstrated that enzymatic crosslinking is an efficient way to obtain fast in situ formation of hydrogels. These dextran-based hydrogels are promising for use as injectable systems for biomedical applications including tissue engineering and protein delivery.[77]

2.2.2 Physical crosslinking methods

Most of the crosslinkers used for covalent crosslinking in hydrogel synthesis may induce toxicity if found in free traces before administration. To overcome this problem purification and verification step is needed. To avoid the use of crosslinking agents by physical crosslinking techniques have been investigated for the designing of hydrogel networks.

2.2.2.1 *Crosslinking by ionic interactions*

Various polymers that can be crosslinked by ionic interactions hence form hydrogels using this method. As covalent crosslinking requires multifunctional molecules as crosslinking

agents, the ionic crosslinking requires multivalent counter-ions as crosslinkers to link polymeric chains. Chitosan based hydrogels were obtained by crosslinking of this chitosan with glycerol-phosphate disodium salt.[78]

Alginate is a well-known example of a natural polymer that can be crosslinked by ionic interactions. It is a polysaccharide that can be crosslinked by calcium ions at room temperature and physiological pH. Alginate gels have been used in both drug delivery and cell encapsulation applications in the beads form usually produced by dripping alginate solution into a $CaCl_2$ bath.[79] For hydrogel preparation, the presence of ionic groups in polymer is not compulsory for ionic crosslinking. For example, dextran, which lacks ionic binding sites for cations, forms a hydrogel in the presence of potassium ions. However, this dextran /potassium gel is unstable in water and therefore is less suitable for drug delivery purposes.[80]

2.2.2.2 *Crosslinking by crystallization*

Apart from ionic interactions or hydrophobic interactions in physical cross-linking of hydrophilic polymers in hydrogel formation, crystallites can act in physical cross-links in block-copolymers and even in homopolymers. The dextran hydrogels were prepared by the process of crystallization. Dextrans are soluble in water, but precipitation was observed in concentrated aqueous solutions of low molecular weight dextran (dextran 6000). The kinetics of the precipitation process showed that the rate of precipitation is accelerated by increase in concentration of dextran solutions, stirring and the presence of salts. Depending on the precipitation time, microspheres or gels were obtained. The precipitates were insoluble in water at room temperature, but readily dissolved in boiling water. IR spectroscopy and modulated differential scanning calorimetry (DSC) demonstrated that the precipitates were crystalline.[81]

A novel hydrogel system in which crosslinking was established by stereocomplex formation between lactic acid oligomers of opposite chirality has been developed. Poly L-Lactic acid (PLLA) and Poly D-lactic acid (PDLA) are semi crystalline materials. Each stereoisomer has a melting temperature of around 170 ºC. Interestingly, in blends of high molecular weight PLLA and PDLA a phase of a higher melting point (around 230 ºC) was observed.[82] This is

attributed to the formation of racemic crystallites, also called stereocomplexes and was first described by Ikada *et al.*[83]

2.2.2.3 C*rosslinking by hydrogen bonds*

A spontaneous formation of hydrogel was observed by mixing of two water-soluble phospholipids polymers, such as poly (2-methacryloyloxyethyl phosphorylcholine-co-methacrylic acid) (PMA) and poly (2-methacryloyloxyethyl phosphorylcholine-co-n-butyl methacrylate) (PMB), in aqueous medium at room temperature without any chemical treatment. The gelation mechanism, effects of ions on gelation and dissolution behavior were determined. The spectroscopic analysis and FT-IR analysis revealed that carboxyl groups in methacrylic acid (MA) formed dimer when two polymer solutions were mixed, and the results of the rheological study showed dissociation of carboxyl groups caused dissolution of the hydrogel. The hydrogen bonds are only formed when carboxylic acid groups are protonated. Thus, the gelation occurred due to the formation of dimers by hydrogen bonding which acts as a physical cross-linking of polymer chains.[84]

Poly (vinyl alcohol) (PVA) hydrogels interacting with DNA mediated by hydrogen bonds (PVA/DNA hydrogel) were developed using ultra-high pressure (UHP) technology. The goal was to create a new method of gene delivery by controlled release of DNA.[85]

Poly (acrylic acid) and poly (methacrylic acid) forms complexes with poly(ethylene glycol). These complexes are held together by hydrogen bonds between the oxygen of the poly(ethylene glycol) and the carboxylic group of poly(meth)acrylic acid, whereas for poly(methacrylic acid) hydrophobic interactions also play a role.[86]

Asymmetric bolaamphiphilic sugar-based crown ether hydrogel were synthesized and their gelation ability with and without alkylammonium ions was investigated. Particularly, the gelation was drastically enhanced by addition of alkylammonium ions, which could result in stabilization due to the intermolecular hydrogen bonding and electrostatic interactions.[87]

2.2.2.4 *Crosslinking by protein interactions*

Another novel method in producing hydrogels involves the crosslinking by protein linkages. It can be either by using genetically engineered proteins or crosslinking by antigen–antibody interactions.

A hydrogel self-assembling method driven by the interaction between recombinant tax interactive protein-1 (TIP1) with the PDZ domain [(PDZ is an acronym combining the first letters of three proteins — post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1)] in a molecule, which is fused to each end of the triangular trimeric CutA protein (CutA-TIP1), and a PDZ domain-recognizable peptide which is covalently bound to each terminus of four-armed poly(ethylene glycol) (PDZ-peptide-PEG). Genetic manipulation based on molecular-dynamic simulation generated a cell-adhesive RGD (Rat Genome Database) tripeptidyl sequence in the CutA loop region [CutA(RGD)-TIP1]. In this way, an approach was developed for in situ hydrogel formation enabling cell entrapment via biospecific interaction between protein and peptide at physiological pH and temperature.[88]

An antigen sensitive hydrogel was prepared by Miyata et al. in which an antigen (rabbit IgG) was grafted to chemically crosslinked polyacrylamide in the presence of antibody as an additional crosslinker. The hydrogel had poor swelling characteristics in the presence of free antigen due to the replacement of polymer-bound antigen, resulting in the release of the antibodies and thereby decreasing crosslink density.[89]

2.3 Characterization of hydrogels

Hydrogels are usually characterized for their morphology, the crosslink density and the structural integrity (porosity, pore size and its distribution), the ultimate capacity to absorb liquids (swelling property) as well as their elasticity. Various techniques have been investigated to investigate the crosslinking interactions among the polymers[90] as could be seen in figure 2.

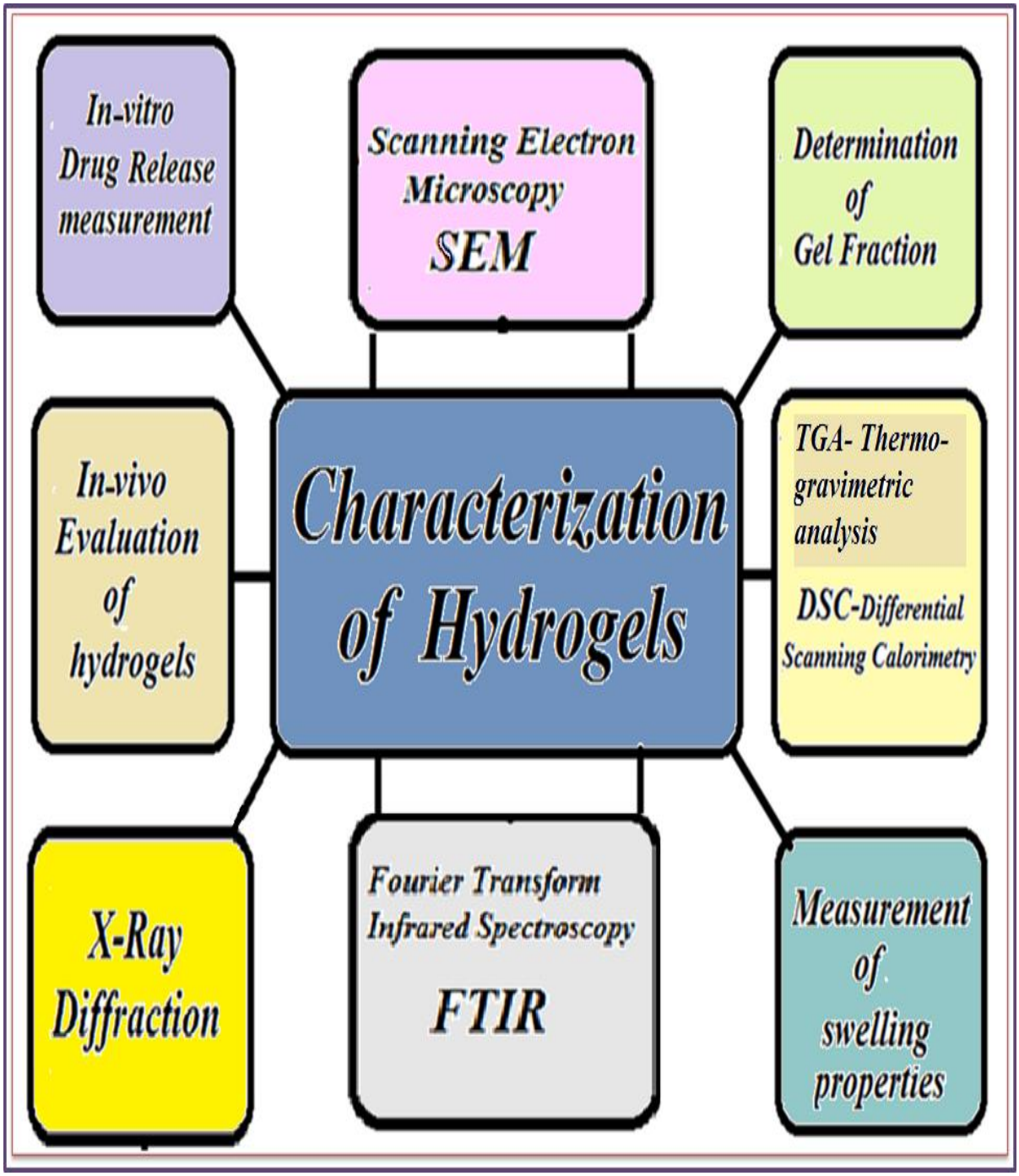

Figure 2. Methods of Characterization of hydrogels

2.3.1 Scanning electron micrography (SEM)

SEM photomicrographs of the polymers are taken in order to investigate and compare their surface morphology. The texture is analyzed by SEM to ensure that hydrogels, such as based on starch, retain their granular structures.[91]

The morphology of the poly (methacrylic acid)/poly (N-isopropylacrylamide) interpenetrating polymeric networks (IPN) was studied with both conventional SEM and cryogenic SEM experiments. Cryogenic SEM was used as a new approach to visualize the IPN morphological behavior in its swollen state. The pH and temperature influence on the IPN morphology was studied. The results showed that a decrease in pH and increase in temperature resulted in a drastic decrease in the pore size of the IPNs.[92]

2.3.2 X-ray diffraction (XRD)

This is another technique used to describe the retention or deformation of the crystalline structure of polymers during the processing pressurization process. Characterization and drug delivery behaviour of starch-based hydrogels prepared via isostatic ultrahigh pressure (IUHP). Szepes *et al.*[91] characterized and investigated the drug delivery behaviour of starch-based hydrogels prepared by ultrahigh pressure. The changes in structure and morphology of potato and maize starches were determined by X-ray diffraction examinations of the samples using D4 Endeavour diffractometer. The crystalline structure of maize starch was sensitive to UHP, so it was changed, while potato starch pressurized in aqueous medium remained stable and retained its original X-ray pattern.

The X-ray diffraction patterns of a copolymer hydrogel of kC-graft-PAAm, k-carrageenan (kC) and acrylamide (AAm) were observed. A considerable modification was noticed in the polysaccharide, leading to a change in molecular association in the formed of hydrogel when compared with kC and AAm.[70]

2.3.3 Magnetic Resonance Imaging (MRI)

MRI uses a powerful magnetic field, radio frequency pulses and a computer to produce detailed pictures at cellular and molecular level. Proton Magnetic Resonance Imaging (MRI) has been used to study the physical changes of hydroxypropyl methylcellulose (HPMC) hydrogels due to microwave irradiation of the polymer. The proton one-dimensional images, derived from relaxation, spin density and diffusion-weighted spin-echo experiments, provide insights on the dynamics of water and the motional state of the polymer inside the hydrogels. The obtained results indicated that the microwave irradiation causes the breaking of the HPMC polymer–hydrogen bonding network in HPMC powder which influences the dynamics of water and polymer chains within its hydrogels.[93]

Proton Magnetic Resonance (PMR) imaging in a thermo reversible gel using Bruker MSL-300 FT-NMR spectrometer measured volume-phase-transition. This was demonstrated in the lower critical solution temperature (LCST) polymer poly (N- isopropylacrylamide) which is swollen in water. The swelling ratios in the axial and radial directions were the same after the thermal collapse.[94]

2.3.4 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy is an established tool for the structural characterization; any change in the morphology of hydrogels changes their IR absorption spectra. The structure and properties of the superabsorbent hydrogels synthesized by graft copolymerization of acrylic acid (AA)/acrylamide (AM)/2-acrylamido-2-methyl-1 propanesulfonic acid (AMPS) onto sodium carboxymethylcellulose (CMC) and montmorillonite (MMT) were evaluated where the intermolecular interaction and morphological change of the hydrogels were characterized by Fourier Transform Infrared (FTIR) spectroscope. It was shown that superabsorbent hydrogel product comprises a crosslink structure of MMT and CMC with side chains that carry carboxylate, carboxamide and sulfate.[95]

The fractions of dissociation of acrylic acid (AAc) units within hydrogel in response to changes in pH and ionic strength of external aqueous solution were determined by FTIR-ATR spectroscopy. The swelling response of hydrogels to the changes in external pH and

ionic strength was governed mainly by the ionic osmotic pressure due to the accumulation of diffusible ions within hydrogels.[96.]

2.3.5 Thermal Analysis

DTA and DSC measure, respectively, the temperature difference and the heat flow difference between a sample and a reference material (subjected to the same temperature variation in a controlled atmosphere). DTA detects any change in all categories of materials, whereas DSC determines the temperature and heat of transformation.

Differential Scanning Calorimetry (DSC) was used to monitor the reaction scheme of arylic based superabsorbing polymers. The heat effects were studied during the polymer synthesis in DSC pan as a micro-scale reactor. Two distinct observations, i.e. inhibition period (IP) and onset of gel formation were recorded during polymerization. By this the effect of reaction temperature and initiator concentration was assessed in the synthesis of superabsorbent hydrogels.[97]

The DSC technique allows studying the drug release and diffusion from a polymeric device to the site mimicking a biological membrane. The drug release from inulin-based hydrogel to a biomembrane model was investigated at pH 4.0 and 7.4 by using DSC that appears to be a suitable technique to follow the transfer kinetics of a drug from a controlled release system to a biomembrane model.[98]

2.3.6 Swelling behavior

The hydrogels were allowed to immerse in aqueous medium or medium of specific pH to know the swellability of these polymeric networks. These polymers showed increase in dimensions related to swelling. A gelatin based pharmaceutical hydrogels, gelled in minutes using oxidized konjac glucomannan (DAK) as a macromolecular cross-linker, and was estimated for equilibrium swelling ratio. From the photographs of the hydrogel in both dry and swollen state, it was determined that the hydrogels remain in the cylindrical form after swelling. However marked volume differences were noticed where the diameter of swollen hydrogel was about 4.0 cm, while the diameter of the dry state hydrogel was only 1.5 cm.[99]

The swelling and deswelling kinetics of poly (N-isopropylacrylamide) (PNIPAAm) hydrogels separately synthesized by means of microwave irradiation and normal water-bath heating. The swelling and deswelling kinetic curves of the PNIPAAm hydrogels were measured in water below and above the lower critical solution temperature (LCST), and their swelling and deswelling kinetic parameters were estimated. Results showed that in comparison to the hydrogel synthesized by the conventional method, the hydrogel synthesized by microwave irradiation had larger swelling and deswelling rate constants as well as lower swelling/deswelling activation energy due to its higher surface area and larger pore sizes, and thus it had faster response behavior.[72]

The swelling responses of pH sensitive psyllium and polyacrylamide based hydrogels were measured in aqueous medium by gravimetric method. The equilibrium percent swelling (Ps) of the polymeric network were calculated as follows:

$$Ps = \frac{W_d - W_s}{W_d} \times 100 \qquad (1)$$

Where W_s and W_d are weights of swollen polymers and dried polymers respectively.[100,101]

2.3.7 Gel Fraction

Gel fraction is mass fraction of the network material resulting from a network forming polymerization or crosslinking process. The gel fraction assays are performed to determine the level of crosslinking, greater the gel fraction, higher is the crosslinking density. If all polymers is in gel fraction (no soluble fraction) and it is completely crosslinked. The degree of cross linking is usually dependent on the molecular weight of the polymers. The polymers with low molecular weight poorly form gel than one with higher molecular weight. Gel fraction was determined in Poly (vinyl alcohol) hydrogels, where Cross-linking does not occur entirely and certain PVA macromolecules remain in the network uncross-linked (sol). The gel fraction *G* in hydrogels was estimated by the formula:

$$G\ (\%) = \frac{W_s}{W_d} \times 100 \qquad (2)$$

Where, W_1 is the weight of the dried cross-linked sample with sol and W_d is the weight of the dried sample after the removing of sol by extraction in water.[102-104]

2.3.8 Porosity

During swelling, the pores located inside the network are rapidly filled with the solvent; atthe same time, the polymer region takes up the solvent from the environment, whose extent depends on the attractive force between the solvent molecules and the polymer segments.[105] Solvent replacement method was used for porosity measurement. Weighed dried discs were immersed in absolute ethanol overnight and weighed after excess ethanol on the surface was blotted using blotting paper. Porosity was calculated using the following equation

$$\text{Porosity } (\%) = \frac{M_2 - M_1}{\rho V} \times 100 \qquad (3)$$

where M_1 and M_2 are the weights of hydrogels before and after immersion in absolute ethanol, respectively. ρ is the density of absolute ethanol and V the volume of gel.[106]

The decrease in density can be attributed to the increase in porosity. The bulk density of dried hydrogels can be determined using picnometer. Certain substances have influence on the density and porosity of the hydrogels. Mahdavinia *et al.*[107] studied the effect of CaCO3 content on the density of the hydrogels. Using the high content of CaCO3 to synthesize hydrogel causes the high number of produced pores, and subsequently the density will be decreased.

2.3.9 Rheology

Hydrogels were evaluated for viscosity under constant temperature of usually 4 °C by using Cone Plate type viscometer when a small amount of material was available. For most of the experiments a flat-plate measuring geometry (acrylic, 4 cm diameter; gap 1 mm) was used. The mechanical strength of the swollen sample of acrylic-based Superabsorbent polymer (SAP) hydrogel was measured by a rheological method. The characterization was conducted

by a controlled strain rheometer at 25ºC. Dependency of the rheological properties of the sample on strain and frequency was investigated.[108]

The rheology measurements yield information of the nature of the water–polymer interaction. The phase transition behavior of water within the chitosan/polyacrylate hydrogels was investigated by means of oscillatory shear rheology. Changes in structure were determined by comparing differences in the rheological measurements at temperatures above and below freezing.[109]

2.3.10 *In-vitro* Release Studies

Since hydrogels are the swollen polymeric networks, interior of which is occupied by drug molecules, therefore, release studies are carried out to understand the mechanism of release over a period of application.[110, 111] *In-vitro* drug release studies can be performed by using diffusion cell method. The release rate of the timolol maleate from the stimuli sensitive hydrogels was determined by the diffusion process. The samples withdrawn were analysed spectrophotometrically at 294 nm for the timolol maleate using Shimazdu Double beam UV-Visible spectrophotometer.[112] First-order model, Higuchi square root time model, Hixson–Crowell model, Weibull distribution, Korsmeyer–Peppas model, etc, are used to evaluate the dissolution profiles of the samples.[113,114]

2.3.11 *In-vivo* Evaluation

In-vivo studies are conducted on prepared optimized hydrogel formulation (test) and on marketed formulation (standard). A wide range of acute, sub-chronic and chronic toxicity studies are conducted, using various routes of administrations in different species. The animal treatment should be complied with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research. The biocompatibility and degradation of the Dacron matrices impregnated with gelatin- chondroitin sulphate (ChS) gels was studied after implantation in subcutaneous pockets in rats. Chemically cross-linked gelatin-ChS gels showed a mild tissue reaction, and almost complete degradation within 18 weeks of implantation. Before *in vivo* implantation, the Dacron samples, as such or impregnated with gelatin or gelatin and ChS, were sterilised by γ irradiation.[115]

In vivo evaluation on the long-term is now necessary to confirm their biocompatibility and establish their life-time. Moreover, the inter-subject variation is more significant in the in vivo study than in vitro skin permeation experiments. It was confirmed by study designed to investigate the *in vitro* and *in vivo* skin absorption of capsaicin and nonivamide from hydrogels.[109]

2.4 Hydrogels for pharmaceutical and biomedical applications

Hydrogels are promising candidates for controlled release devices, bioadhesive devices, or targetable devices of therapeutic agents. The excellent hydrophilic properties, high swelling ratio and biocompatibility, have led them widely applicable in biomedical / pharmaceutical area as antibacterial materials, tissue engineering, biosensors and sorbents for the removal of heavy metals.[116-120] These water-swollen, crosslinked biomedical materials are efficient carriers for the development of novel pharmaceutical formulations for the delivery of drugs (peptides and proteins), as targeting agents for site specific delivery and as components for the preparation of protein or enzyme conjugates. They have gained considerable existence in drug delivery through parenteral, ocular, rectal, vaginal, dermal and nasal routes.[121, 122]

2.4.1 Transdermal drug delivery

Water-based polymeric gels offer several advantages over traditional oleaginous bases in terms of ease of application, cosmetic acceptability (colorless and water-washable) and desirable drug release characteristics.[123] Drug delivery to the skin has been traditionally conducted for topical use of dermatological drugs to treat skin diseases, or for disinfection of the skin itself. In recent years, a transdermal route has been considered as a possible site for the systemic delivery of drugs. The possible benefits of transdermal drug delivery include that drugs can be delivered for a long duration at a constant rate, that drug delivery can be easily interrupted on demand by simply removing the devices, and that drugs can bypass hepatic first-pass metabolism. Furthermore, because of their high water content, swollen hydrogels can provide a better feeling for the skin in comparison to conventional ointments and patches. Versatile hydrogel-based devices for transdermal delivery have been proposed so far.[122]

The hydrogel membranes mainly composed of three kinds of latex particles within carboxymethyl cellulose (CMC) matrix were prepared for the purpose of transdermal drug release. These microgels were poly (acrylic acid-cosodium acrylate), poly (acrylic acid-co-2-ethylhexyl acrylate) and poly(N-isopropyl acrylamide), which were developed to give the membrane a higher swelling ratio, a better adhesive property and a thermo-responsive behavior, respectively. Gel particles of PNIPAAm or its copolymers underwent deswelling above lower critical solution temperature (LCST), creating more space for the membrane to absorb more water and in turn increasing the swelling ratio. Also, they simultaneously expelled caffeine to the highly swollen CMC matrix, thus increasing the caffeine-release rate.[124]

A transdermal system for delivering selegiline using a hydrogel-based drug reservoir and a rate-controlling membrane (Solupor polyethylene membranes) was designed. Both the R- and S-forms of selegiline were examined in this study to elucidate the stereoselectivity of skin to selegiline. The experimental results suggested that Solupor can be used as a substrate to control the permeation of selegiline. The amount of drug permeating across the skin can be reduced by the membranes.[125]

Hydrogels are used for local drug delivery in the control of wound healing. Nanocomposite hydrogel wound dressing was prepared using combination of polyvinyl alcohol hydogel and organoclay, i.e. Na-montmorillonite. The results showed that the nanocomposite hydrogels could meet the essential requirements for the reasonable wound dressing with some desirable characteristics such as relatively good swelling, appreciated vapour transmission rate, excellent barrier against microbe penetration and mechanical properties.[126]

2.4.2 Orally administered hydrogels

The orally administered hydrogels are used for peroral and oral drug delivery and drug delivery. It leads the drug to mouth (oral cavity), stomach, small intestine, or colon. Drug delivery to the oral cavity can have versatile applications in local treatment of diseases of the mouth, such as periodontal disease, stomatitis, fungal and viral infections, and oral cavity cancers. Long-term adhesion of the drug containing hydrogel against copious salivary flow, which bathes the oral cavity mucosa, is required to achieve this local drug delivery. A

bioadhesive tablet or hydrogel-based ointment can also be utilized for the topical treatment of certain diseases in the oral cavity. Chitosan-based hydrogels have been recognized as excellent candidates for oral delivery due to their mucoadhesive properties. Chitosan hydrogels have been developed for the local release of a number of other drugs in the oral cavity. In addition to the released drugs, the chitosan polymer itself has shown antifungal activity. For instance, chitosan hydrogels and films were able to limit adhesion of the common pathogen Candida albicans to human buccal cells.[127]

The Chitosan based matrix has been used as a reliable colonic controlled-release system for the release of 5-aminosalicylic acid (5-ASA) or diclofenac sodium (DS) and introduced into enteric-coated capsules for controlled release to the colon.[128] Alginate-N, O-carboxymethyl chitosan hydrogels with calcium for oral delivery of protein drugs to different regions of the intestinal tract e.g., for duodenal targeting, small intestine targeting, or colon targeting.[129]

Colon specific hydrogels of polysaccharides have been specifically designed because of presence of high concentration of polysaccharidase enzymes in the colon region of GI (gastrointestinal) tract. Drugs loaded in such hydrogels showed tissue specificity and changed in the pH or enzymatic actions that cause liberation of drug.[130]

2.4.3 Ocular delivery

In comparison to other ophthalmic formulations such as suspensions and eye ointments, the hydrogels may offer better feeling, with less gritty sensation to patients. In particular, in-situ-forming hydrogels are attractive as an ocular drug delivery system because of their facility in dosing as a liquid and their long-term retention property as a gel after dosing.

Fast forming hydrogels prepared by crosslinking a poly (ethylene glycol) (PEG)-based copolymer containing multiple thiol (-SH) groups were evaluated for the controlled ocular delivery of pilocarpine and subsequent pupillary constriction. A strong correlation between pilocarpine release and pupillary response was observed. In conclusion, the current studies demonstrate that in situ forming PEG hydrogels possess the viscoelastic, retention and sustained delivery properties required for an efficient ocular drug delivery system.[131]

In situ forming poly (ethylene glycol) (PEG)-based doxycycline hydrogels were developed and evaluated for their wound healing efficacy in rabbit corneas in organ culture. The doxycycline-PEG hydrogels accelerated corneal wound healing after vesicant injury offering a therapeutic option for ocular mustard injuries. Histology and immunofluorescence studies showed a significant reduction of matrix metalloproteinase-9 (MMP-9) and improved the healing of vesicant-exposed corneas by doxycycline hydrogels compared to a similar dose of doxycycline delivered in phosphate buffered saline (PBS, pH 7.4).[132]

2.4.4 Subcutaneous delivery

Biocompatibility is a prerequisite requirement to overcome undesirable body responses, such as inflammation, carcinogenicity and immunogenecity. Due to their high water content, hydrogels are generally considered as biocompatible materials. They also cause minimal mechanical irritation upon in-vivo implantation, due to their soft and elastic properties and possess broad acceptability for individual drugs with different hydrophilicities and molecular sizes; and unique possibilities (crosslinking density and swelling) to manipulate the release of incorporated drugs.[133,134]

Some of these may offer an advantage for the delivery of certain delicate drugs, such as peptides and proteins. The high-strength injectable Pluronic hydrogels were synthesized by enzyme-mediated cross-linking for controlled drug delivery. They showed controlled erosion, bio-adhesion, thermo-sensitivity, and injectable properties, ideal along with *in situ* depot formation in the tissue. They can possibly be utilized for sustained delivery of therapeutic proteins, genes, and chemical drugs.[135] The poly (β-amino ester) (PAE) as a duo - functional group for synthesis of the novel sensitive injectable hydrogel is used for controlled drug/protein delivery. Furthermore, the cationic nature of PAE is utilized to make the ionic complexes with anionic biomolecules loaded into the hydrogel such as insulin.[136]

The hydrogels could target drugs to specific body sites and control the release of drugs for prolonged periods of time. For this they can be used as promising drug delivery systems for treating various types of cancers. Injectable chitosan hydrogels have been synthesized for localised cancer therapy using paclitaxel as a model drug.[137]

2.4.5 Rectal and vaginal delivery

The bioadhesive characteristics of hydrogels make them a valuable way to overcome the shortcomings of conventional suppositories, such as uncontrolled drug release and insufficient retention in the rectum. Once in the rectal cavity, the development of mucoadhesiveness would help to immobilize the hydrogel for a required period of time thus prolonging the drug release for local or systemic use.

A thermosensitive hydrogels was formulated which was based on poloxamer 407, a thermosensitive polymer, and hydroxypropylmethylcellulose (HPMC), a bioadhesive polymer, intended for the rectal delivery of quinine in children. In consideration to the therapeutic applications, the formulations should be in the liquid state at ambient temperature (generally 25 ºC), should gel at body temperature (37 ºC) and should be adhesive on the rectal mucous membrane.[138] The temperature sensitive and mucoadhesive pluronic-based hydrogels were designed, which were capable of retaining their rheological and mucoadhesive properties after dilution with vaginal fluids. It enables them as promising formulations for the vaginal administration of drugs.[139]

2.4.6 Hydrogels for tissue engineering

When designing suitable biomaterials for tissue-engineering applications, biological and chemical parameters are frequently taken into account. The hydrogels, due to their swelling properties, can be made to resemble the physical characteristics of soft tissues. Hydrogel materials also generally exhibit high permeability and good biocompatibility making these materials attractive for use in cell encapsulation and tissue engineering applications. Due to the biocompatibility, permeability and physical characteristics, hydrogels are good candidates as biomaterials for use in many medical applications, including tissue engineering. Hydrogels may be useful for manipulation of tissue function or for tissue regeneration or replacement. The use of photopolymerization in the preparation of hydrogels is advantageous in comparison with conventional crosslinking methods because liquid hydrogel precursors can be delivered and crosslinked to form hydrogels in situ in a minimally invasive manner such as by injection. This process also gives one spatial and temporal control over the conversion of a liquid to a gel, so that complex shapes can be fabricated.[140]

Cellulose is a major extracellular matrix component of plant cells, composed of poly (1,4´ -anhydro-b-β-D-glucopyranose). Being an abundant naturally occurring polymer, it is renewable, as well as biodegradable and derivatizable for a large number of biomedical and pharmaceutical applications. Cellulose based materials including bacterial cellulose are of increasing interest in tissue engineering. They have been utilized in wound healing,[141] artificial kidney, artificial blood vessels, bone, cartilage and cardiovascular tissue engineering etc.[142-146]

2.5 DRUG

Captopril, (2S)-1-[(2S)-2-Methyl-3-sulphanylpropanoyl] pyrrolidine-2-carboxylic acid an angiotensin-converting enzyme (ACE) inhibitor was chosen as a drug to be loaded into the hydrogel formulation. Its chemical structure is shown in the figure.

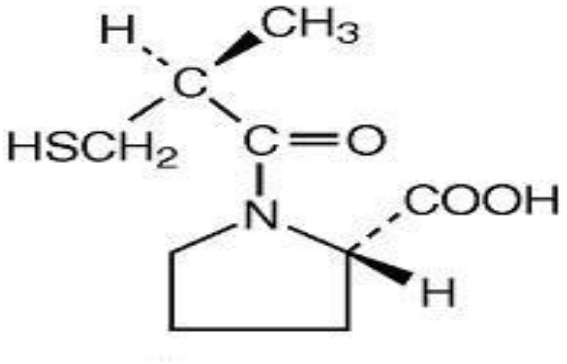

Figure 3.Chemical structure of captopril
(C9H15NO3S, MW = 217.3)

It is widely used as an antihypertensive drug and for the management of congestive heart failure because of its effectiveness and safety. It has low toxicity and commonly prescribed patients suffering from chronic illness. Therefore, a long term therapy is needed for their treatment. Commercially, it is available as immediate release tablets of 12.5- 50 mg. It is available in market with following brand names.

Table 1. Commercially Available brands of Captopril

Brand names	Manufacturers
Capoten	Glaxosmithkline
Captil	Werrick Pharmaceuticals
Capoten	Bristol-Myers Squibb
Captolane	Sanofi-Aventis
Captoril	Benson
Acepril	BMS (United Kingdom)
Acepress	BMS (Italy)
Cesplon	Esteve (Spain)
Dilabar	Qualigen (Spain)

Captopril has been a drug of choice in hypertension management and also effectively used for congestive heart failure. There are almost 25% people among the world's population suffering from hydpertension.[147,148] Captopril is successfully used for hypertensive patients but its antihypertensive effect remains for a period of 6–8 hours. For maintaining minimum effective concentration three to four CAP administrations are required. For this reason, usually 25-100 mg drug is needed for successful medication of hypertension and heart disorders. In some conditions, for therapy of heart failure an initial dose of 6.25 mg is needed three times a day. Therefore, development of a controlled release drug delivery system for CAP would be beneficial; hence once daily formulation will be needed to achieve significant effects, providing an optimum drug concentration, minimizing drug fluctuations and adverse drug effects.

Captopril is a suitable candidate for controlled release drug delivery systems due to its short elimination half-life of less than 2 hours.[149] However, it is not easy to develop an oral controlled release formulation of captopril, due to its *in-vitro* and *in-vivo* stability concerns. The drug being freely soluble in water and physiological aqueous solutions may undergoes the problems like dose dumping as well as burst phenomena from controlled/sustained release drug delivery systems.[150] Moreover, its bioavailability is also affected in presence of food and higher intestinal pH.

Captopril has been formulated in both rapid and slow release drug delivery systems either for its fast availability into blood circulation to attain peak plasma concentration in lesser time or to be available for longer period of time. For its instant availability, sublingual tablets were prepared, which was an effective way for lowering the arterial blood pressures in emergency situations. Hence, there were rapidly available therapeutic levels of drug as compared to other orally administered captopril tablets.[151]

In treating hypertension and heart failure long term management is required by antihypertensive drugs. In this context, there is a need to develop controlled release dosage form, where dissolution rate should be controlled. For this reason, various researchers have prepared the controlled release formulations such as floating matrix tablets, gastroretentive systems, microcapsules, and nanoparticles. Martinez et al prepared the *in-vitro* sustained release floating tablets of captopril by using metalose SH 4000 SR/ Sodium bicarbonate. The floating tablets were prepared to prolong the gastric residential time and to avoid the degradation by higher pH of intestine.[152] Polymeric matrices were formulated to control the release rate of captopril using various polymers such as, Hydroxypropyl methyl cellulose (HPMC), Sodium carboxy methyl cellulose (NaCMC) and ethyl cellulose (EC). This work was concerned to study the effect of polymers and surfactants on release of captopril.[153]

In another work, the captopril was entrapped in albumin based biodegradable microparticles formulated by Dandagi *et al.*[154] by using emulsification- heat stabilization technique. Its *in-vitro* analysis had shown the drug release upto 24 hours and *in-vivo* evaluation proved its successful targeting to lungs, liver, kidneys and spleen. Similarly Cellulose propionate (CP) microparticles were prepared by solvent evaporation technique for controlled release of captopril. They were proven as useful products in gradually decreasing systolic blood pressure throughout 24 hour time period in comparison to reference solution.[155] Captopril-loaded microspheres have also been prepared by solvent evaporation method, using Methocel and Eudragit RS as release-controlling factors and to evaluate captopril release. Microspheres and micropellets of captopril have also been prepared with different polymers (chitosan, ethyl cellulose, sodium alginate and Hydroxy propyl methyl cellulose) through the techniques of microencapsulation. Polyacrylamide-co-methylcellulose (PAAMA-co-MC) hydrogels loaded with captopril were prepared and evaluated for their controlled release

characteristics. The sustained releases of captopril from cyclodextrin nanoparticles have also been investigated.[156]

As already mentioned that the hydrogels are not only overcomes the shortcomings of conventional dosage forms but also have advantages over other controlled release formulations like microparticles and matrix tablets. Because of their cross-linked polymeric network they have ability to entrap drugs and protect them from unfavorable physiological environments. Captopril loaded into hydrogel formulations may remain stable and released for longer periods of time.

2.5.1 Physical properties

Captopril is a white or almost white crystalline powder. These white crystals melt at about 88°C, which recodifies and melt again at 105°C or 106°C. It is freely soluble in water, in methylene chloride and in methanol and also dissolves in dilute solutionsof alkali hydroxides. It has a characteristic sulfide-like odour.[157,158] Captopril is not hygroscopic under ordinary conditions. Above 40°C, captopril shows extraordinarily high water solubility.[159]

2.5.2 Captopril Stability

In solution, this product undergoes an oxygen facilitated, first order, and free radical oxidation at its thiol to yield captopril disulfide. Hydrolysis at the amide linkage occurs only under forcing conditions. Oxidation is delayed using lower pH, chelating agents, higher concentration, degassing, minimizing headspace, and incorporation of antioxidants. No degradation is seen in methanol (40µg/ml) for up to 2 weeks at 5 °C. It shows maximum stability below pH 4; however in the absence of oxygen it remains stable at relatively higher pH values. [160] Captopril contains a sulphydryl group that results in its self-dimerization, rapid formation of disulfide conjugates with endogenous thiol-containing compounds (cysteine, glutathione) and plasma protein binding. Therefore, during *in-vivo* evaluation, the detection or measurement of free captopril concentration needs molecule derivatization or an addition of chemical stabilizer in biological or in vitro samples to prevent captopril disulphide formation. Various Flouresence or UV active agent such as N-(1-pyrenyl) maleimide (NPM) and p-bromophenacyl bromide (p-BPB), have been used as chemical stabilizers. The formation of Captopril disulfide can be controlled by lowering the pH below 4, adding

chelating agents (EDTA) or antioxidants. Dithiothreitol (DTT) added to the plasma samples successively reconstitutes captopril converted to disulfide, by increasing free thiol content from human serum albumin.

2.5.3 Pharmacokinetics

Captopril, after oral administration, is rapidly absorbed from stomach and small intestine. Approximately 75% of orally administered captopril is absorbed in fasting conditions. After oral ingestion of a single dose the maximum blood level and therapeutic effect is observed after 45–90 min. By presence of food in stomach Bioavailability is reduced to 25-30%, therefore the drug should be administered one hour before meal. When enters into blood, nearly 25% of captopril is binds to plasma protein. Captopril is partly undergoes metabolism and about 95 % of the absorbed drug is excreted in urine, where 40-50 % is unchanged and the remaining is captopril disulfide.[161]

2.5.4 Clinical Uses

Therapeutic benefits of Captopril are due to its vasodilatory effects as well as inhibition of some renal function activities. The following are its main uses in various clinical conditions:

i) A widely acceptable and drug of choice for treating Hypertension.

ii) It has been approved by FDA for the treatment of cardiac disorders like congestive heart failure, left ventricular dysfunction and post-myocardial infarction.

iii) Captopril has an additional advantage to preserve the function of kidneys during diabetic nephropathy. The effect of captopril on creatinine clearance, protein-urea and metabolic controlled in diabetic neuropathy were determined. In addition of controlling blood pressure alone, captopril provides protection against deterioration of renal function in patients with insulin dependent diabetic nephropathy.[162]

iv) Captopril also possesses mood elevating characteristics and has shown in some patients. This is indicative of its antidepressant effect. However, it has not been reported in formal clinical trials.[163]

v) Additionally, this compound has also been investigated to possess cytotoxic activity for use in the treatment of certain types of cancer such as in treating lung tumor. The impact of this drug on cell proliferation, apoptosis, and angiogenesis has been evaluated.[164]

2.5.5 Adverse drug reaction (ADR)

1) The adverse drug reaction (ADR) profile of all ACE inhibitors is almost similar to one another. Captopril like other ACE inhibitors commonly cause cough, which is attributed to increase in bradykinen plasma level.[165]
2) Unlike most of the other ACE, captopril usually causes rash.
3) Due to oral administration of this drug, taste alterations have been noticed in many cases. It may impart a characteristic metallic taste and in some situations it leads to taste loss. This effect is associated with sulfhydryl group present in Captopril.[166]
4) Other adverse effects of captopril include proteinuria, angioedema, hyperkalemia, tetragenicity, postural hypertension, agranulocytosis, leukopenia and in some cases it may be responsible to acute renal failure.[167]

2.6 Excipients and Formulations

In this research work, two polymers and two monomers were used which are given as following:

The polymers used were:

i. Hydroxypropyl methylcellulose (HPMC) and
ii. Polyvinyl Alcohal (PVA),

The monomers were:

i. Acrylic acid and
ii. 2-acrylamido-2-methylpropane-sulfonic acid (AMPS).

They were cross-linked in different combinations and proportions for preparing three types of hydrogel formulations.

I. Hydroxypropylmethylcellulose-g-polyvinyl alcohol-co-poly(acrylic acid)

II. Polyvinyl alcohol-co-poly (\2-acrylamido-2-methylpropane-sulfonic acid)
III. Hydroxypropylmethylcellulose-g-poly (Acrylic acid-co-2-acrylamido-2-methylpropane-sulfonic acid)

The method used for developing the above polymeric network was free radical polymerization, using potassium persulfate (KPS) as initiator and N, N-Methylene-bis-acrylamide was used as crosslinking agent in a hydrothermal water bath. The hydrogel formulations were also synthesized under influence of microwave radiations. A pH- sensitive hydrogel semi-IPN hydrogel formulation was prepared by crosslinking of HPMC with PVA and acrylic acid. It was prepared to protect the drug from exposure to unfavourable physiological conditions and enables drug release for longer periods of time. The other formulation was a gastroretentive hydrogel comprising of PVA and AMPS to avoid any instability of captopril at higher intestinal pH. Moreover, a superabsorbent hydrogel was synthesized by using HPMC, acrylic acid and AMPS, to release the drug throughout its passage from gastrointestinal tract. Hence, the drug would be available at both lower and higher pH.

2.6.1 Polymers and monomers

Polymers are macromolecules with molecular weights, consisting of repeated units of monomers. Monomers are polymerized by different techniques and results in formation of polymers. A large number of polymers of different grades have been used and are being used in the development of various pharmaceutical formulations, such as coated tablets, matrix tablets, microcapsules and hydrogels. They can be natural polymer (cellulose, carrageenan, collagen, guar gum, alginate etc) synthetic polymers (polyvinyl alcohal, polyvinyl pyrollidone, polyethylene glycol, polyethylene oxide, polymethacrylate, polyacrylic acid etc) as well as semisynthetic polymers (Hydroxypropyl methylcellose, Carboxymethylcellulose, methyl cellulose, chitosan etc). Usually, the semi-synthetic polymers are derived from natural sources. The polymers derived from natural origins are more biocompatible with physiological envoirnment. Cellulose and its derivatives are widely accepted among research groups because of their eco-friendly properties. Cellulose can be degraded by several microorganisms (bacteria and fungi) in air, soil and water.[168]

Cellulose-based polymeric networks can be synthesized either by using chemical agents or high energy radiations such as electron beam, gamma radiations or even microwave radiations. The selection of chemical agents (Cross-linkers and initiators) depends upon the characteristics of cellulose derivatives such as aldehydes, urea derivatives, multifunctional carboxylic acids and epichlorhydrin. Usually the crosslinking occurs in water or aqueous solutions but in some cases it also occurs in dry state such as crosslinking of cellulose by polycarboxylic acids via condensation polymerization without water and requires reasonably higher tempratures.[169,170] In comparison to crosslinking reactions involving chemical agents, use of high energy radiations have attracted the attention of researchers as it is easily controlled and involves lesser amounts of chemical agents. Radiation induced crosslinking has an additional advantage in biomedical research fields as they sterilize the product. In this way, crosslinking and sterilization occur simultaneously; therefore, use of radiations is beneficial with respect to health and environmental safety. High energy radiations cause scission of polymer chains and many cellulose derivatives can be crosslinked under influence of mild intensity of radiation. It can take place in both solid form as well as aqueous solution of polymers.[171-174] The crosslinking is dependent upon amount of polymer and radiation dose that can either be intensity or exposure time. The microwave heating of many polymers has influence on their properties such as cellulose derivative; Hydroxypropyl methylcellose (HPMC) used in food production makes a question about effect of radiation on polymer.

2.6.1.1 Hydroxypropyl methylcellose (HPMC)

Hydroxypropyl-methylcellose (HPMC) or Hypromellose is a white or slightly off-white tasteless, odourless powder. It is soluble in water and when dissolved, it forms a viscous colloidal solution.[175-177] It is semisynthetic, viscoelastic and inert non-ionic cellulose ether. In solution, it does not react with salts or metals and there is no intake of ionic charges. Hence, it does not exhibit any sort of reaction during and after preparation of Pharmaceutical preparations. One of its other significant characteristic is its stability in both acids and alkaline medium, even for long term storage. The aqueous solutions of HPMC are resistant to enzymatic degradation and the formed products have better stability than material produced by starch, dextrin and other natural polymers.[178,179]

HPMC is one of the most successfully used polymers in preparing different oral drug delivery systems.[180,181] In pharmaceutical industry, it is used in manufacturing of both coated and controlled release matrix tablets. Depending on its grade, it can be either used as binder or as a component with ability to prolong the release of medicinal agent in digestive tract. Moreover, it is commonly used as lubricant in ophthalmic preparations and has numerous applications in cosmetic industry and in many other commercial products. It also serves as structural and storage material in plants.[182-185]

HPMC comprise of polymeric backbone of cellulose and during its synthesis cellulose fibers are heated with a caustic solution, which are then reacted with methyl chloride and propylene oxide. The obtained product is fibrous material, which is finally purified and ground to a fine, uniform powder. HPMC is a linear polymer comprising of repeated units of glucose which are joined together by β-1, 4 glucosidal bonds with the functional groups (–OH, -CH_3, and -$OCH_2CHOHCH_3$) attached to basic unit of HPMC. Additional networks of hydrogen bonds link the polymer chains with one another.[186]

Hydroxypropyl methylcellose consists of functional groups in various ranges, 3-12% of Hydroxypropyl groups and 19-30% of methoxyl group (-OCH_3). Its chemical name is Propylene glycol ether of methylcellulose Hydroxypropyl methylcellulose.[187] Its structural formula is presented in the figure 4.

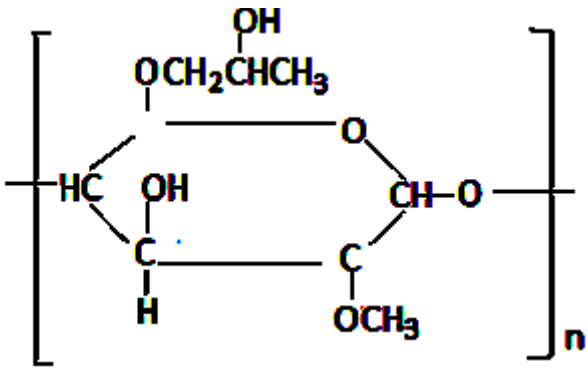

Figure 4. Chemical structure of HPMC

Hydroxyproplymethylcellulose is propylene glycol ether of methylcellulose. As illustrated from its chemical structure in figure, that substituent 'R' can either be –H or –CH_3 as well as –$CH_2CH(CH_3)$ OH. Because of different proportions of methoxyl and hydroxypropyl

groups in the structure of polymer, it is available in numerous grades, which are categorized according to USP in terms of measuring viscosity in 2% aqueous solution at 20°C by using a Ubbelodhe type of viscometer. The other way to differentiate HPMC grades is on basis of thermal gelation temperature by drying at 105°C for 2 hours. The determination of apparent viscosity specifies the chain length of polymer and categorizes it into HPMC of lower or higher viscosity grades.[188]

The HPMC polymer on exposure with water swells and forms a viscous, colorless and transparent gel product. Due to its high swelling capability, it has an important effect on the release of loaded/ incorporated drug. The water or biological fluids diffuse the formulation that causes relaxation in polymer chain and consequently volume expansion occurs.[189] This resultantly leads to release of entrapped drug from polymeric device.

The mechanism of drug release from pharmaceutical drug delivery systems containing HPMC is dependent upon their designing and particular characteristics. In dry systems such as matrix tablets, there is low coefficient of diffusion. On the other hand, in highly swollen systems i.e. hydrogels, diffusion coefficient is of high magnitude as in case of pure water. Upon contact with water, the HPMC swells leading to changes in polymer and the drug diffuses out of the hydrogel formulation.[188]

HPMC has been used by many research groups in preparing different controlled release formulations such as matrix tablets, microspheres and hydrogels. Hydroxypropyl Methylcellulose Matrices containing naproxen and naproxen sodium were prepared, their structure and hydration characteristics were determined.[190] In that work, the effect of molecular weight of polymer on the drug release mechanism was measured. Carbamazepine loaded HPMC and Chitosan microspheres were prepared by spray drying technique, where different grades of low, medium and high molecular weight polymers were used to study their effect on release profile.[191] HPMC has been used in preparing alginate/hydroxypropyl-methylcellulose gel beads, which were loaded withbovine serum albumin (BSA). HPMC was successful in making a suitable carrier system for controlled release of albumin, by improving its release rate in physiological saline solution.[192] In another work HPMC was grafted with acrylic acid with aim of obtaining copolymeric hydrogelprepared by in situ emulsifier-free emulsion polymerization using benzyl peroxide as an initiator.[193] Davaran *et*

al.[194] prepared HPMC hydrogels with polyethylene glycol (PEG), which were found to be a promising drug delivery system for delivering 5-amino salicylic acid to the colon for treatment of ulcerative colitis. Thermo-Sensitive HPMC/PNIPAAm Hydrogel were prepared for oral drug delivery of 5-fluorouracil. Being a thermo-responsive., the swelling behavior of the formed hydrogels was dependent upon temperature changes, where swelling ratios reduced with increasing temperature. It was also concluded that drug release was dependent on the amount of HPMC in the prepared hydrogel.

2.6.1.2 Poly(vinyl alcohol) (PVA)

Polyvinl alcohal was discovered in 1924, and since that time it has been widely used in variety of scientific researches due to its desireable characteristics such as its bio inertness and non-carcinogenicity.[196] It is a synthetic polymer composed of 1,3-diol linkages [-CH2-CH(OH)-CH2-CH(OH)-] or 1,2-diols [-CH2-CH(OH)-CH(OH)-CH2-]. This variation in its microstructure depends upon the polymerization conditions for vinyl ester precursor. Due to stability concerns polymer is not simply formed by polymerization of vinyl alcohol; firstly, polyvinyl acetate is prepared by polymerization of vinyl acetate and then converted to polyvinyl alcohol.[197] Its structural formula is given below in figure 5.

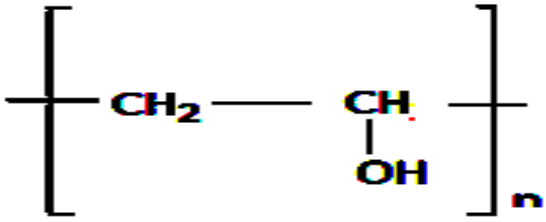

Figure 5. Chemical structure of PVA,
Molecular formula: (C2H4O) n

Polyvinyl alcohal is available in both powdered and granular form. It is white or creamy white in color and possess no odour. Its melting point ranges from 220 °C - 240 °C. It is a water soluble polymer, but practically insoluble in organic solvents. However, it is soluble in water at room temprature, but its solubility in water is increased at higher temperatures. PVA 4% solution at room temperature has viscosity range of 35-50 cp. In FTIR spectrum

Polyvinyl alcohal shows characteristic peaks at wavenumber (cm^{-1}) 3300, 2941, 1456, 1329, 1142, 1089, 921, 844 and 620.[198, 199]

Due to its flexibility and high tensile strength, it has numerous applications in pharmaceutical industry and biomedical fields. It has an excellent adhesive, film forming and emulsifying characteristics. It exhibit resistance to solvents, grease and oil as well as also to act as barrier to oxygen and aroma. PVA has Poisson's ratio between 0.42 and 0.48, means it is close to incompressible.[200]

Studies on the mechanism of dissolution and changes in crystallinity and swelling behaviour of PVA and its physical gel-forming capabilities, have been carried out.[201,202] PVA has various applications such as in making controlled drug delivery systems, membrane preparation, transdermal patches, tendon repair, contact lenses as well as stabilizer in emulsions. PVA is used as viscosity enhancer in ophthalmic preparations for prolonging the contact time of drug with eye.[203-206] Due to biocompatibility of PVA, its hydrogels have been reported to resist cell adhesion and adsorption of protein.[207, 208] PVA hydrogels have been formed by various techniques like physical crosslinking using freeze-thaw processes, using chemical agents (initiator and crosslinker), or physical treatments by radiations. The most common synthesis of PVA hydrogels is free radical crosslinking involing the use of various crosslinking agents such as glutaraldehyde, N, N'- Methylene bis-acrylamide (MBAAm), etc. However, PVA hydrogels crosslinked with acrylic acid have also been prepared by microwave radiations. Rapid synthesis of three dimensional PAA/PVA network was prepared in aqueous solution by this technique.[109-211]

The swelling behavior and drug release mechanism of poly(vinyl alcohol) hydrogel have been investigated by Varshosaz et al. The kinetics of swelling and drug release depending on amout of polymer as well as crosslinking density and percentage of drug loading were studied. The effect of structural properties of polymeric network on drug release was also determined.[212] The use of photo-crosslinkable PVA hydrogels as tissue engineering scaffolds has been studied bySchmedlen et al. because of their mechanical properties, elasticity and tensile strength of PVA hydrogels they can be effectively utilized in soft tissue applications. They are preferred over PEG photopolymerizable hydrogels due to availability of more

active sites for bioactive molecules. The mechanical characteristics of PVA are dependent upon number of functional groups available for crosslinking.[213] Cavalieri *et al.*[214] prepared hydrogel microparticles based on cross-linking of poly (vinyl alcohol) and methacrylate PVA-MA, in presence of dextran T70 to evaluate the capability of formed microdevice for drug delivery. Doxorubicin was loaded as model drug and its cytotoxic effect on colon cancer cells was analyzed. Electro-responsive polyvinyl alcohol (PVA) hydrogels were prepared by solution-casting using glutaraldehyde as crosslinking agent. They were loaded with sulfosalicylic acid and studied the effect of electric field on the rate of drug release.[215]

2.6.1.3 Acrylic acid (AA)

Acrylic acid also commonly known aspropenoic acid is widely used monomer in hydrogel preparations. It is synthesized from propylene, that is well known gaseous byproduct of oil refineries, Althouth, it is not the basis of acrylic acid synthesis but it can be referred as derivative of ethylene in which one of hydrogen atom has been substituted with carboxylic group. There also exist natural sources of acrylic acid as it has been detected in rumen fluid of sheep and also produced by some species of marine algae.[216] The chemical structure and formula of acrylic acid are presented in figure 6.

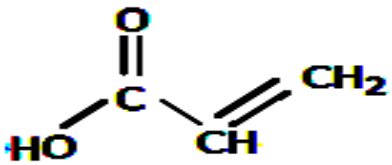

Figure 6. Chemical structure of Acrylic acid

Acrylic acid serves as a basic substance for its various derivatives such as acrylamine, acrylonitrile and acrylic esters. At room temperature, glacial acetic acid is transparent and colourless liquid miscible with water, possessing sharp penetrating odour resembling the odour of acetic acid. It freezes to crystalline form at lower temperature; hence it should be stored above its melting point i.e. 13.5 °C. It has a boiling point of 141°C at 101kPa and density 1.045 g/ml at 25 °C. Acrylic acid can be explosive as it has tendency to polymerize spontaneously. Therefore certain measures should be adopted during its storage, most

importantly is selection of suitable container of glass, ceramic or stainless steel and using a polymerization inhibitor (hydroquinone monomethyl ether).[217,218]

Several precautionary measures should be adopted while working with acrylic acid monmer because of its toxic and irritating effects. Acrylic acid causes irritation and has corrosive action on skin and the respiratory tract. High and prolonged exposure results in pulmonary edema. Eyes should be protected as it can cause severe and irreversible injury of eyes. Acrylic acid, after oral administration in rats has LD50 value of 340 mg/kg.

Acrylic acid has number of industrial applications, where its primary use, accounting for is in the production of resins and acrylic esters (about 67% of its total usage), that are utilized in coatings and as adhesives. One of its most common and important application is in producing superabsorbent polymers. Other uses are in oil treatment and water treatment chemicals.[219] On other side, it has a demerit in creating environmental pollution. The acrylic acid released to the environment usually enters the water resources. Due to its miscibility with water and its vapor pressure, its removal is not possible remains in water. From atmosphere it can be cleared by rain and if comes to contact with soil, it enters surface or ground waters.[220]

Acrylic acid monomers polymerize by reacting at their double bonds and form high molecular weight homopolymer known as polyacrylic acid. Acrylic acid also combines with its derivative monomers (acrylamides, vinyl, acrylonitrile, butadiene and styrene) and resultantly produces copolymers. These homopolymers and copolymers are utilized pharmaceutical industry and daily life such as, in manufacturing coatings, plastics, paints and adhesives. Poly (acrylic acid) is brittle, hygroscopic and colorless solid having glass transition temperature 106°C. At high temperature range of 200-250°C, it loose water and become crosslinked water insoluble polymer anhydride. It finally decomposes at temperature approximately 350°C.[218] Poly(acrylic acid) (PAA) or Carbomer is a well-known ionic, hydrophilic, water permselective polymer. It has higher affinity to physiological medium of colonic mucosa than for mucosal tissue in stomach or small intestine.[221,222] PAA is an anionic polymer, hence at pH 7 or above its side chains will lose their protons and attain negative charge. By this phenomenon, the polymer will be able to absorb water, many times of its original weight.

Poly-acrylic acid and its copolymeric hydrogels have been successfully used as carriers in controlled drug release technology because of their biocompatibility, biodegradability and many other unique characteristics. In addition, the pH responsive nature of acrylic acid polymers makes them a promising candidate in colon specific drug delivery and controlling the drug release for longer period.[223] PAA hydrogels are fragile, in order to add strength PAA is polymerized and crosslinked with other polymers. Acrylic acid has been crosslinked with all kinds of polymers (natural, synthetic or semisynthetic), resulting promising drug carriers.. Shin et al prepared an interpenetrating polymer network (IPN) hydrogels with dual characteristics, exhibiting both pH responsiveness as well as thermosensitivity. It was synthesized by crosslinking poly (acrylic acid) (PAAc) with poly(vinyl alcohol) (PVA) and incorporated with indomethacin.[224] Kadłubowski *et al.*[225] prepared hydrogels by photocrosslinking of poly(acrylic acid) (PAA) with polyvinylpyrrolidone (PVP). The resulting polymeric network was a pH-sensitive drug delivery system hydrogels for glucose oxidase. In another work, acrylic acid was crosslinked with a cellulose derivative, carboxymethyl cellulose. An acrylic acid/carboxymethyl cellulose (AA/CMC) superabsorbent hydrogel was prepared in aqueous solution by glow-discharge electrolysis plasma, using N,N′-methylenebisacrylamide (MBA) was used as a crosslinking agent.The superabsorbant was senitive to pH and salt concentration, and a reversible swelling and deswelling behavior was investigated.[226]

2.6.1.4 2-Acrylamido-2-methylpropane sulfonic acid (AMPS)

2-Acrylamido-2-methylpropane sulfonic acid (AMPS) is a highly reactive sulfonic acid acrylic monomer. Figure 7 shows chemical structure of AMPS.

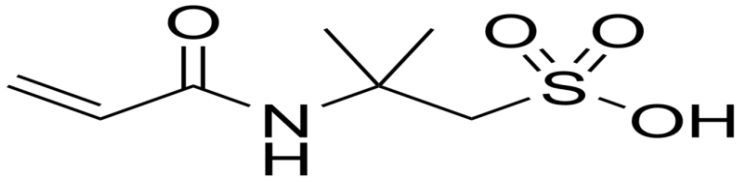

Figure 7. Chemical Structure of AMPS,
Molecular formula, $C_7H_{13}NO_4S$

It is prepared by reaction of acrylonitrile, oleum and isobutylene, in aqueous solution.The AMPS monomer is a propanesulfonic acid having substitution at the C_2 position with a

methyl group and an acrylamido moiety. It contains an unsaturated vinyl group and a terminal sulfonic acid, which are its reactive sites. AMPS is also available as its sodium and ammonium salts which are formed by its neutralization with sodium hydroxide or ammonium hydroxide, respectively.[227]

AMPS monomer appears as white crystalline powder or granular particles having water possessing hygroscopic character. The melting point of this solid has been determined as Melting point 195 °C (383 °F; 468 K). Its density is 1.1 g/cm3 (15.6 °C) and soluble in water having solubility of about 1×106 mg/L at 25°C.[228, 229]

In recent years, 2-Acrylamido-2-methylpropane sulfonic acid (AMPS) has attracted the attention of pharmaceutical and other scientific researchers because of its thermal stability, resistance to hydrolysis, water solubility and ability to impart a remarkable swelling behavior in hydrogels. Its thermal and hydrolytic stability is due to the presence of sulfomethyl and dimethyl groups which in combination create a steric hindrance to amide functionality. This high swelling character is attributed to its highly ionizable sulfonate groups that make it a strongly hydrophilic compound.[230-234] Dissociation of AMPS is not dependent upon specific pH; hence AMPS derived hydrogels are able to swell at all pH ranges.[235,236] However, these hydrogels exhibit higher swelling ratio at lower pH in comparison to neutral or alkaline pH.

Due to the presence of vinyl group in AMPS, it can be polymerized or crosslinked under the influence of high energy radiations. Increasing the concentration of AMPS in reaction medium provides more reactive vinyl groups, consequently, higher radiation assisted polymerization could occur. Therefore, crosslinking during AMPS based hydrogel formulation depends upon content of AMPS, which causes an increment of ionizeable functional groups resulting higher electrostatic repulsion that leads to expansion of polymeric network system.[237] After hydrogel synthesis, unreacted residual monomer can be easily removed by washing with water.[238]

It is synthetic monomer used in combination with number of polymers altering their physicochemical characteristics. AMPS has been used in preparing hydrogels by crosslinking with broad range of synthetic polymers, natural polymers, derivatives of natural polymers as well as with other monomers such as acrylamide (AAm) and acrylic acid. These hydrogels

have applications in biomedical fields like controlled release drug delivery and superabsorbants for removal of heavy metal ions from aqueous solutions. Sulfonic acid in present in AMPS has ability to ionize completely in aqueous solutions. Due to this characteristic, AMPS inhibits the undesirable precipitation of mineral salts including iron, aluminum, calcium, magnesium, barium, zinc and chromium.AMPS/Polyvinyl alcohol (PVA) hydrogels have been synthesized by gamma radiation.[239] In another work, Carboxymethyl cellulose (CMC) was crosslinked with AMPS along with other monomers.[95] Durmaz *et al.*[240] prepared and characterized 2-acrylamido-2-methyl-1-propane sulfonic acid -co- acrylic acid (AMPS/AA) hydrogels. They were synthesized by radical polymerization in the presence of N,N′-methylenebisacrylamide (MBA) as the crosslinking agent using potassium persulfate (KPS) as initiator.

2.6.1.5 Potassium persulfate (KPS)

Potassium persulfate (KPS) also named as potassium peroxydisulfate is an inorganic compound. This salt is an odorless, white solid powder with a molar mass 270.322 g/mol and melting point 1067°C. Its density is 2.477 g/cm3 and is highly soluble in water with solubility of 5 g/100 mL at 20 ºC. It is commonly used as initiator to initiate polymerization reactions. It can be synthesized by electrolysis of cold solution of potassium hydrogen sulfate in sulfuric acid under influence of high current density.[241,242]

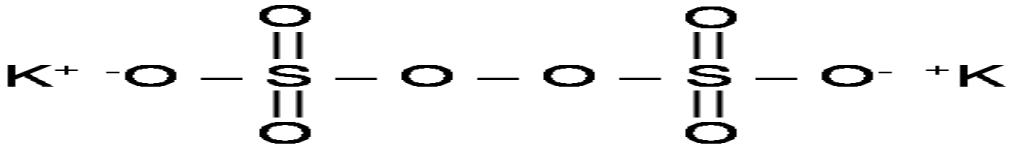

Figure 8. Chemical Structure of Potassium persulfate , Molecular formula= K2S2O8

Potassium persulfate is a thermal initiator widely used in polymerization or crosslinking process for preparing hydrogels. KPS and other thermal initiators possess high activation energy (125-160 kJ•mol-1), therefore the polymerization reactions are usually occur at higher temperatures ranging from 60ºC to 90ºC.[243] The initiation of polymerization is first step of hydrogel synthesis, during which there is formation of an active center that further starts polymerization in monomer units or crosslinking of polymers. In this process, firstly the

initiator creates radicals, which are then transferred to monomer, resulting in generation of polymer chain. All kinds of initiators cannot be used for every monomer and polymer. For example, radical initiators are best suited for initiation on carbon-oxygen double bond in aldehydes and ketones as well as carbon-carbon double bond of vinyl monomers.[244] As initiator KPS has been used in many polymerization processes such as in preparing polytetrafluoroethylene and styrene-butadiene rubber, which are important commercially available materials.[245] It has been utilized in preparing hydrogels for drug delivery and other useful scientific procedures. Acrylic acid, acrylamide, methacrlyic acid and many other monomers as well as polymers have been polymerized and crosslinked, respectively.[246-249] Being an oxidizing agent, KPS is used in oxidation of many products in organic chemistry, for instance in oxidation of phenols.[250]

2.6.1.6 N,N'-Methylenebisacrylamide (MBAm or MBAA)

N,N'-Methylenebisacrylamide is a white crystalline powder, very slightly water soluble with water solubility of 0.01-0.1 g/100 mL at 18 ºC. It is incompatible with strong acids, strong bases and strong oxidizing agents. It is a cross-linking agent used for formation of polymers and in crosslinking of polymers in hydrogel synthesis. Bisacrylamide is used for SDS-PAGE in biochemistry because it is one of the compounds of polyacrylamide gel.[251] The chemical structure of MBA is shown in figure 9.

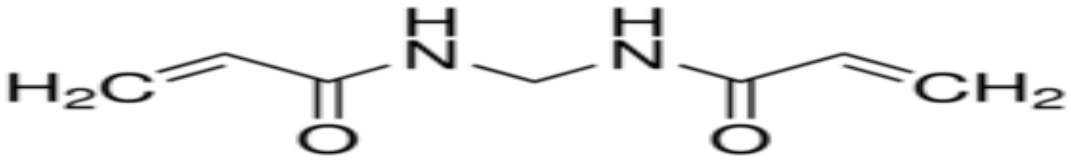

Figure 9. N,N'-Methylenebisacrylamide (MBAm), Molecular formula $C_7H_{10}N_2O_2$

Bisacrylamide polymerizes with various monomers, among them one of the commonly used is acrylamide. MBAm is capable of creating cross-linkages between polyacrylamide chains, therefore; creating a strong polymeric network rather than unconnected linear chains of polymer. An optimum concentration of MBA should be used for polymerization and crosslinking due to its stability concerns. It has been investigated that by using very low concentration of crosslinker, its methylene group by hydrolytic degradation is converted to

formaldehyde. By using crosslinker concentration above 0.3- 0.6 %, crosslinking density was increased and degradation was controlled for several days (upto 10 days). Further increasing the content to 1% or above, it remained stable and no degradation was observed for longer periods.[252,253] N,N-methylenebisacrylamide has been used as crosslinking agent for preparing biomedical hydrogels with potential for utilization controlled drug release or enhancing mechanical strength of bone substitutes.[254] Ray *et al.*[255] prepared pH sensitive poly (acrylic acid-co-acrylamide)/MBA nanosized hydrogel colon-specific delivery of 5-fluorouracil. The hydrogel drug delivery systems, as those based on N-isopropylacrylamide (NiPAAm) and itaconic acid (IA), were prepared by using N,N-methylenebisacrylamide (MBA) as crosslinking agent.The concentration of MBA used was ranging from 2.0- 4.0 wt.% with respect to monomer concentration. The hydrogels were successfully synthesized by MBA, loaded with Lipase and its controlled release was investigated under mild conditions. These hydrogels were responsive to both temperature inside tract and pH of its media.[256,257]

Aims and Objectives

In Pharmaceutical technology, the research study has objectives related to betterment of the health care system to improve the quality of life. The goals of this study are briefly discussed as below:

1) The aim of this work is to detect the possibility of using mixtures of polymers as prolong drug release systems using Captopril (CPT).
2) This will determine the compatibility and usefulness of the polymer combinations being used in Hydrogel formulation.
3) The formulation will be prepared using a Radical graft polymerization technique and evaluated by *in vitro* and *in vivo* studies.
4) This research project will contribute to the rational drug therapy.
5) This will be a promising study to ensure the safe and efficacious administration of the drug providing more effective treatment. A suitable fraction of drug will be available for treatment, thus minimizing the adverse drug reactions related to conventional drug delivery systems.
6) This research study will also have a contribution towards cost effective treatment.

Chapter no. 3

Synthesis of Hydroxypropyl methylcellulose-graft- poly (vinyl alcohol)-co-poly(acrylic acid) hydrogels for the Controlled Release of captopril and its *in-vitro* Evaluation

Summary

Background of the Study

A microwave induced irradiation synthesis, was proposed for the preparation of Hydroxypropyl methylcellulose-graft-poly(vinyl alcohol)-co-poly (acrylic acid) hydrogels for the controlled release of Captopril, an antihypertensive drug.

Methods

The hydrogels were separately synthesized by using microwave irradiation method and conventional water-bath heating method. Chemical groups, thermal stability and surface morphology of these hydrogels were characterized by FT-IR, DSC and SEM. Swelling ratios of the gels were measured gravimetrically in the pH range from 1.2 to 7.4.

Results

Micrographs obtained from scanning electron microscopy (SEM), revealed that the gels synthesized using microwave irradiation had more porous structure; therefore, they had higher swelling ratios in comparison to hydrogels synthesized by water-bath method. Thermal analysis (DCS and TGA), FTIR and XRD determination had confirmed the formation as well as stability of the new polymeric network.

Conclusion

A stable network of Hydroxypropyl methylcellulose (HPMC), poly (vinyl alcohal) (PVA) and acrylic acid was developed in shorter time period under influence of microwave radiations. The formed hydrogels could be an efficient drug carrier for controlled delivery of captopril for hypertension management.

Keywords: Hydroxypropyl methylcellulose, poly(vinylalcohal), acrylic acid, microwave radiation, captopril

3.1 Introduction

The crosslinking of polymeric materials prevents the dissolution of hydrophilic polymer chains / segments into the aqueous environment.[258,259] These swellable polymeric materials have been widely recognized as the stable carrier for drug delivery systems, because of their ability to simulate biological tissues. Hydrogels being biocompatible materials have been investigated to protect the drug, from *in-vivo* environment e.g, enzymatic degradation, unfavorable pH or other hostile conditions.[33]

Development and designing of multi-component polymeric blends has become the subject of great interests because such materials possess an efficient hybrid performance superior to their individual components.[260] In various hydrogel formulations more than one polymer are crosslinked to form semi-interpenetrated polymer network (semi-IPN). Semi-IPN is a technique of formulating a stable polymeric network of at least two polymers with an additional non-covalent interaction.[261] It is attractive in producing synergistic properties from the component polymers to provide combined physical and mechanical properties to the hydrogels for use in drug delivery. When a hydrophilic gelling polymer is interpenetrated with a relatively hydrophobic gelling polymer, the resultant IPN hydrogel is expected to have an improved capability of immobilizing a drug. Hydrogels based on natural polymers exhibit poor mechanical strength, which limits their usefulness.[262] Hence, blends of judicially selected natural and synthetic polymers over comes this short coming by improving the strength. It combines the biocompatibility of biological component with physical and mechanical properties of the synthetic components. A combination of natural and synthetic polymers has been found to be useful in enhancing the release of short half-lived drugs under physiological conditions. Grafting of vinyl monomers onto natural polymers such as cellulose and its deivatives has been widely accepted.[263-265]

To improve the synthesis of hydrogel as well as their properties, many researchers tried to use a variety of novel methods, including e-beam radiation, microwave radiation, photo-initiated polymerization and plasma-induced grafting. The radiation crosslinking of polymers avoid the use of additional chemical reagents as they have ability to crosslink water-soluble polymers in their aqueous solution, without using initiators or using low quantity of initiator.[266-268] In cases of biomedical applications, it allows the simultaneous synthesis and

sterilization of the product. High-energy radiation causes the generation of radicals that leads to scission on the polymer chain. This has been also investigated for cellulose and as well as its derivatives.[269-271] Microwave radiation possess special heating energy, having significant advantages over the conventional thermal methods for preparing hydrogels. Due to the rapid and uniform penetration properties, microwave energy can instantaneously absorbed and directly heat the entire volume of a material.[272] The microwave irradiation has been successfully used in recent years for preparing useful polymeric devices for drug delivery. In a research work, a guar-g-polyacrylamide hydrogel was prepared by microwave radiations, without using the radical initiator.[273] In another work, the temprature sensitive hydrogels were prepared separately by microwave irradiation and conventional water bath heating method. Both formulations were characterized by FT-IR, DSC and SEM and compared by the results, which showed that microwave radiations shortened the preparation time, improves the yield and swelling.[72] Similarly, PVA/PAA hydrogels were prepared, where acrylic acid was modified with PVA in the synthesis of polymeric matrix under the influence of microwave radiations.[211] The effects of varying power and exposure of microwave radiations have been investigated in the grafting of methyl methacrylate on bamboo cellulose.[274]

Hydroxypropyl methylcellulose (HPMC), one of the most widely used cellulose derivative as hydrogel-forming polymer with numerous industrial applications such as, in the production of coated and controlled release tablets, as a component of body lotions, ointments etc. It also serves as structural and storage material in plants.[275, 185]

Polyvinyl Alcohol (PVA) possesses the strength, high water retaining ability, long-term temperature and pH stability. It is polymerized and crosslinked with other monomers and also with polymers to strengthen the hydrophilic systems.[276]

Acrylic acid (AA) has been extensively used monomer in hydrogel synthesis because AA is relatively economical and easily polymerized to a higher molecular weight polymer[277] by various formulation techniques.

In this work, microwave irradiation method, is used for hydrogels synthesis. The hydrogels were synthesized respectively by microwave irradiation method and conventional

hydrothermal method. The porous structures of the hydrogels synthesized using two different methods were compared, and discussed the effects of hydrogel structures on its swelling.

3.2 MATERIALS & METHODS

3.2.1 Chemicals

Hydroxypropyl-methyl cellulose (2600-5600 cps), PVA, 99% hydrolyzed, Mw, 85,000 - 124,000 (Aldrich, product of USA), Acrylic Acid (Sigma Aldrich-Netherlands), N, N-Methylene-bis-acrylamide, 98% (Fluka-Switzerland), Potassium persulphate (AnalaR, BDH-England), Potassium dihydrogen phosphate (Merk- Germany), All the solvents used were of analytical grades. Deionized distilled water was obtained from our laboratory.

3.2.2 Preparation of Hydrogel

The hydrogel was prepared by free radical polymerization, using thermostatic water bath and microwave raditions

3.2.2.1 Method using Thermostatic Water Bath

Various quantities of polymers (HPMC and PVA) were added in distilled water and stirred at 80ºC for 1 h. Then the HPMC solution subjected to nitrogen purging for about 30 min and potassium persulfate (0.5% W/W) was added to initiate the reaction by generating free radicals. After that the reactants was cooled down to 30ºC and MBA as cross-linking agent dissolved in acrylic acid (AA) was added under magnetic stirring. Final volume was adjusted by addition of deionized distilled water. After that, the above mixture was poured in test tubes and heated in water bath at 50°C, 55°C, 65°C and 75°C for 30min, 1h, 2h and 3h, respectively. Then, the tubes were cooled to 25°C and hydrogels were taken out and cut in the form of discs of nearly 8mm long. They were then thoroughly treated with ethanol and distilled water mixture (50:50) for removing catalysts and uncross-linked monomer till the pH of solutions after washing becomes nearly same as before being used. After washing process, the hydrogel discs were air dried for overnight and then transferred to oven at 45°C for 4 to 5 days until they attain a constant weight.

3.2.2.1.1 Hydrogel Formulations prepared using different concentrations of Acrylic acid and crosslinking agent

The following were the suitable quantities of acrylic acid and crosslinker (MBA) to be further used in the formulations as shown in table 1 and table 2, respectively. Among these three M_2 was selected on the basis of visual appearance and stability of discs during swelling. After selecting the appropriate concentration of acrylic acid, the concentration of crosslinking agent was varied. MBA, 1% of monomer concentration was considered to show better results and stability.

Table 1. Hydrogel Formulations using different concentrations of Monomers

Formulation Code	Polymer (HPMC and PVA, 50:50)g/100g	Monomer (Acrylic acid) g/100g	Crosslinker MBA, mol% of Monomer
M_1	2	20	1
M_2	2	15	1
M_3	2	10	1

Table 2. Hydrogel Formulations using different concentrations of Crosslinker

Formulation Code	Polymer (HPMC and PVA, 50:50) g/100g	Monomer (Acrylic acid) g/100g	Crosslinker MBA, mol% of monomer
C_1	2	15	0.5
C_2	2	15	0.75
C_3	2	15	1
C_4	2	15	1.25

3.2.2.1.2 Hydrogel Formulations using different proportions and concentrations of Polymers

Both polymers (HPMC and PVA) were used in various ratios. HPMC, a cellulose derivative was crosslinked with PVA to form semi- IPN as shown in table 3. Among these

formulations, the ratio of these polymers used in P1 was consdidered more suitable on the basis of swelling. Hydrogels with varying polymer concentration are presented in table 4.

Table 3. Hydrogel Formulations using different ratios of PVA and HPMC

Formulation Code	Polymer (2g/100g) (HPMC:PVA)	Monomer (g/100g) (Acrylic acid)	Crosslinker(MBA), mol% of Monomer
P1	3:1	15	1
P2	2:1	15	1
P3	1:1	15	1
P4	1:2	15	1
P5	1:3	15	1

Table 4. Hydrogel Formulations using different concentrations of Polymers

Formulations by Conventional Thermostatic water bath method			
Formulation Code	Polymers, g/100g (HPMC and PVA, 3:1)	Monomers, g/100g	Crosslinking agent, mol % of monomer concentration
F1	0.5	15	1
F2	1	15	1
F3	1.5	15	1
F4	2	15	1
F5	2.5	15	1
F6	3	15	1

3.2.2.2 Hydrogel formulation prepared by Microwave Radiation

The above mentioned traditional heating method was used that let the reaction to take place in the thermo-stated water bath for longer periods of time. On the other hand, rapid synthesis

of Hydrogels was performed by using microwave radiations. In order to make comparison of both methods, same proportions of polymers and monomers were taken. The whole mixture was placed in Electrolux domestic microwave oven and it was first irradiated by electrical power of 100 W for 5 minutes. Then, after an interval of one minute, it was further exposed for 5 minutes at 180 W and finally, after one minute interval, the material was treated for another 5 minutes with maximum power output set at 300 W. After that, the hydrogels formed were cut into discs and treated with ethanol/ water mixture as previously described in the above method.

Crosslinking reactions during hydrogel formation involve chemical or physical interaction among functional groups of the components. In present work, crosslinking of both polymers (HPMC and PVA) with acrylic acid in the presence of crosslinking agent (MBA) could be presented as shown in figure 1.

Hydrogels prepared using different ratios of polymers and monomers by microwave radiation) have been presented in table 5. The hydrogel formulations synthesized using conventional water bath have been donated by ‘F’, while polymeric networks developed under influence of microwave radiations are named as ‘R’. The ‘R’ hydrogels were prepared with the same ratio of components as that for ‘F’ hydrogels, involving exposure to different doses of radiations.

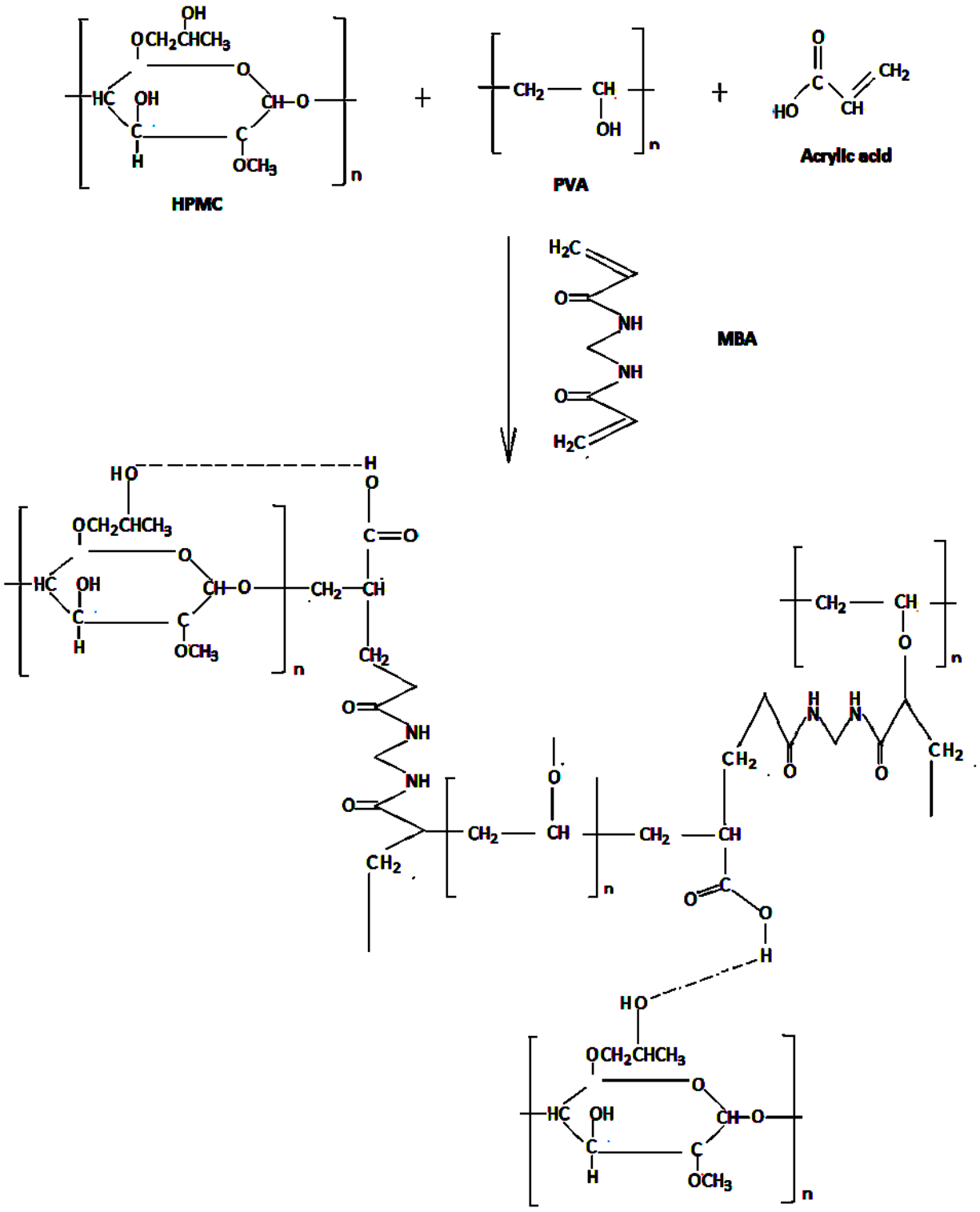

Figure 1. Crosslinked HPMC-g- PVA-co Poly (AA) Hydrogel

Table 5. Hydrogels using different concentrations of Polymers and radiation dose

Formulations by Microwave Radiations				
Formulation Code	Exposure time at 300W (min)	Polymers (HPMC and PVA) g/100g	Monomers, g/100g (Acrylic acid)	Crosslinking agent, mol % of monomer concentration
R1	5	0.5	15	1
R2	5	1	15	1
R3	5	1.5	15	1
R4	5	2	15	1
R5	2.5	1.5	15	1
R6	7.5	1.5	15	1
R7	10	1.5	15	1

3.3 *In vitro* Evaluation

3.3.1 Fourier Transform Infrared Spectroscopy (FT-IR*)*

FT-IR spectrophotometer (Bruker, Tensor 27) was used to record the spectra of hydrogel, HPMC, acrylic acid and AMPS. The hydrogel samples were ground by the help of cutter as well as pestle and mortar. The components and crushed hydrogel samples were then analysed in wavelength range of 4000 to 500 cm^{-1}.

3.3.2 Scanning Electron Microscopy (SEM)

SEM images were taken to investigate the surface morphology of super-absorbent hydrogels using a scanning electron microscope (Quanta 250, FEI). Both drug free formulations and drug loaded samples were ground and scanned at different magnifications to observe the microscopic surface of dried hydrogels. It is therefore performed, to assess the capability to adsorb and entrap the drug into their polymeric network.

3.3.3 X-Ray Diffraction (XRD)

X-Ray Diffraction analysis determines the crystallinaty and amorphous properties of the substances. It investigates the interaction of components or polymers and drug. Xpert Pro diffractometer (Panalytical) diffractometer used to record x-ray diffraction. The XRD patterns of pure drug and drug loaded formulation were measured at room temperature by scanning at angle 5-50° (2 Theta), scanning speed of 20/ min-^{1}.

3.3.4 Thermal analysis

Thermal analysis was recorded by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) using Q5000 series (TGA instruments) and Q2000 series (TA instruments), respectively. The hydrogel samples were crushed into powder form using pestle and mortar and passed through a mesh no. 50. For measuring TGA, 1- 4 mg of ground sample was placed in platinum pan connected to microbalance and heated upto 500°C at a rate of 20°C/min in nitrogen atmosphere. To record DSC, hydrogel samples (1 to 3mg) along with HPMC, AMPS and acrylic acid were placed in aluminum pan crimped with an aluminum lid and heated from 0-500°C at the same rate used for TGA.

3.3.5 Swelling Study

The swelling of hydrogels was measured at different pH (1.2, 4.5 and 7.4) at room temperature. Dried discs of hydrogels were accurately weighed and immersed in swelling medium i.e. 0.1 M USP phosphate buffer solution. Hydrogel discs were weighed at regular intervals of time and before weighing they were placed on filter paper to remove excess of solution from the surface. The hydrogels were weighed for a period until they attain equilibrium. The swelling ratio was calculated as:

$$S = \frac{w_s}{w_d} \qquad (1)$$

Where, w_s is the weight achieved after swelling and w_d denotes the weight of dry hydrogel discs. The percentage equilibrium swelling was determined by equation given below:

$$\% ES = \frac{w_{eq} - w_d}{w_{eq}} \qquad (2)$$

Where, w_{eq} is the equilibrium weight and w_d is the initial weight of hydrogels before swelling study.

3.3.6 Drug loading

Hydrogels were loaded with drug (captopril) using absorption method by immersing the dry discs of hydrogels in 100ml captopril solution (1% w/v) comprising of phosphate buffer solution and methanol (50:50). The dics were swollen till they achieved equilibrium, then taken out and dried in oven at 40°C to their constant weights. The amount of drug loaded in hydrogels was measured by extracting them with the methanol/ phosphate buffer solution in the same ratio used for drug loading. The extraction was done repeatedly at regular intervals and each time with freshly prepared solution until no drug remains in the extracting solution. All samples of drug solutions used during extraction procedure were analyzed for drug contents. The caliberation curve of captopril was drawn by preparing its various dilutions to determine the drug concentration spectrometrically at λ_{max} of 205nm. The amount of captopril loaded in hydrogels was calculated by following relation.

$$\text{Amount of drug} = WD - Wd \qquad (3)$$

Where *WD* and *Wd* represents weights of dried hydrogel discs after and before immersion in drug solution, respectively.[17]

3.3.7 Determination of Gel Fraction

Dried hydrogels were extracted at room temperature in distilled water for 7 days. The extraction process was done to remove any sol that may be present in the hydrogel. The extracted hydrogels were then dried again in the oven at 60ºC until a constant weight is achieved. The percent gel fraction (%G) will be calculated using the equation as below:

$$\%GF = \frac{W_e}{W_i} \times 100 \qquad (4)$$

where w_i and w_e are the dry weight of the sample before and after extraction, respectively.

3.3.8 Drug Release

Drug release measurement was carried out by dissolution process using 0.1 M USP phosphate buffer solutions of lower and higher pH values (pH 1.2 and pH 7.4). The dried hydrogel discs loaded with captopril were placed in 500 ml buffer solution (dissolution medium) maintained at 37°C, agitated by a paddle stirrer at a speed of 50 rpm. Then, the samples were taken out at specific time intervals and drug released was measured by UV-spectrophotometer at λ_{max} of 205nm.

3.3.9 Drug release kinetics

Drug release models were used to determine the mechanism of drug release as given below:

Zero order kinetic models

It relates the drug delivery systems, where the rate of drug release does not exhibit concentration dependency. It is represented as:

$$M_0 - M_t = Kt \qquad (5)$$

Where, M_0 isthe initial quantity of drug, M_t is the fraction of drug released at time t and K is proportionality constant.

First order kinetic models

The first order kinetics describes the concentration dependent release of drug and is represented by the following equation:

$$Log\ M_0 - Log\ M_t = K_1 t/2.303 \qquad (6)$$

Where, M_0 is the initial amount of drug, M_t is the drug concentration released at time t and K_1release constant.

Higuchi Model

Higuchi model can be presented by a simplified equation as:

$$Q = K_H t^{1/2} \qquad (7)$$

Where, Q represents the fraction of drug released at time *t* and K_H is Higuchi constant.

Korsmeyer- Peppas model

Korsemeyer-Peppas model is described by a simple empirical equation to describe the Fickian and non-Fickian drug release from polymeric drug carriers, given as following:

$$M_t/M_\infty = K\,t^n \qquad (8)$$

Where, 'K' is kinetic constant that incorporates the geometric and structural properties of the hydrogels and other drug carriers. M_t/ M_∞ represents the drug fraction released at time *t* and *n* is release exponent. When the value of n is 0.45, it indicates Fickian release order and for n = 1, represents case II transport mechanism. On the other hand, n value between 0.45 and 1 corresponds to non- Fickian diffusion.

Weibull model

The dissolution and release process was described by an equation expressing the fraction of drug accumulated 'M' in dissolution medium at time *t* given as:

$$M = 1 - \exp\left[\frac{-(t - T_i)^b}{a}\right] \qquad (9)$$

Where, *a defines* the dependency on time, *b* denotes the shape parameter of dissolution curve and the other parameter 'T_i' represents the lag time before dissolution process.

3.4 Results and Discussions

3.4.1 FT-IR

The structure and formation of crosslinkage among the polymers were investigated by spectra recorded using FT-IR spectroscopy. In figure 2, spectrum of HPMC (a) shows an absorption band at 3444.60 cm^{-1} is assigned to stretching frequency of the hydroxyl (-OH) group. Another band at 1373.63 cm^{-1} is due to bending vibration of –OH. Other stretching vibration bands related to C-H and C-O were observed at 2929 cm^{-1} and 1055.52 cm^{-1}, respectively. The noted peaks of pure HPMC were similar to observations of Wang *et al.*[278]

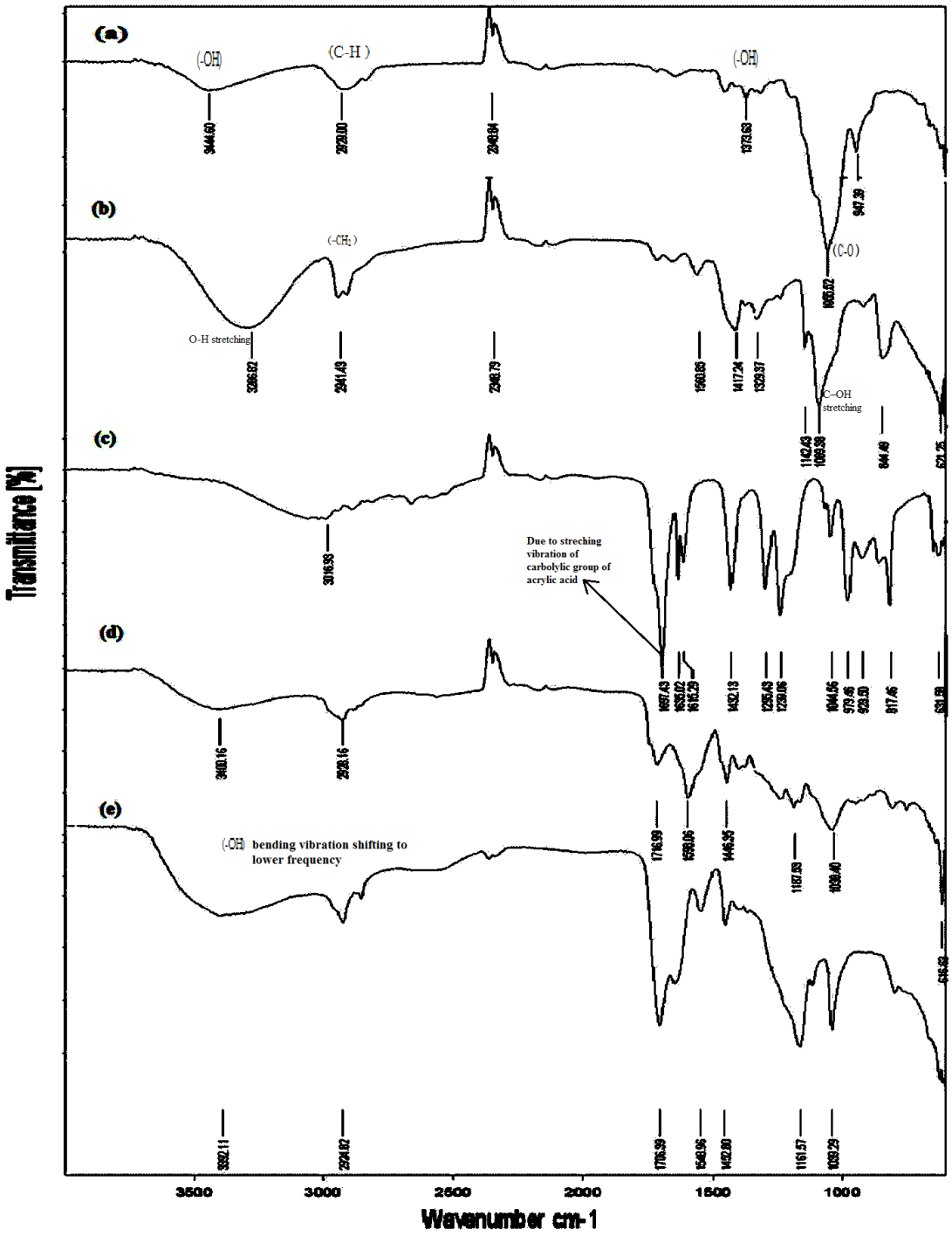

Figure 2. FT-IR Spectra of HPMC (a), PVA (b), Acrylic acid (c) HPMC/PVA-co- AA hydrogels, F1 (d) and R1 (e)

In PVA's FT-IR spectrum (b), at 2941.43 cm^{-1} stretching $–CH_2$ groups in alkanes, 1089.38 cm^{-1} characteristic C–OH stretching, and a wide-strong absorption peak at 3286.82 cm^{-1} due to O-H stretching was observed.

The spectra of HPMC-g-PVA-co-AA polymeric network suggests the formation of intermolecular hydrogen bonding due to carboxylic acid groups of acrylic acid as observed by the appearance of an absorption peak at 1716.99 cm^{-1} in (d) and 1706.39 cm^{-1} in (e) . These peaks noted were similar to peaks observed by Debajyoti *et al.*[279] and Pillai *et al.*[280]

In figure 2 the hydrogel spectrum (d) and (e) indicates the shifting of -OH vibration band of HPMC from 3444.60 cm^{-1} to 3400.16 cm^{-1} and 3392.11 cm^{-1}, respectively. This was observed due to formation of hydrogen bonds confirming that -OH groups of HPMC and PVA have reacted with -COOH groups of acrylic acid.

Hydrogels prepared by both methods, (conventional water bath and microwave radiation) exhibited OH- stretching due to hydrogen bonding and carboxylic vibrations and CO- because of intramolecular bonding in the same regions. At the same time, it could be clearly seen that the FTIR spectra shapes of the hydrogels R and F were similar. It suggested that the use of microwave irradiation method could successfully crosslink the HPMC/PVA-co-acrylic acid polymeric network.

3.4.2 SEM

Scanning electron microscopy (SEM) was performed to study the morphology of hydrogels. Figure 3 shows fractured surface morphology of drug loaded and drug free hydrogels. The surface roughness and porosity of both types of hydrogels (F and R) could be compared from their SEM images. From the SEM micrographs of surfaces of the air-dried gels R and F, it can be seen that the surface of gel F was denser and rather smooth containing few cavities and small spherically shaped protrusions. In comparison, the surface of R hydrogel formulation is rough with some deep and interconnected pores. In comparison to F hydrogel, the R hydrogel formulation had more uniformly porous network structures as shown in figure 3 (B). The uniformity in porosity was due to rapid and instantaneous penetration of microwave energy throughout the surface.[272] Hence, R hydrogels would facilitate the diffusion more easily into hydrogel matrix during swelling process.

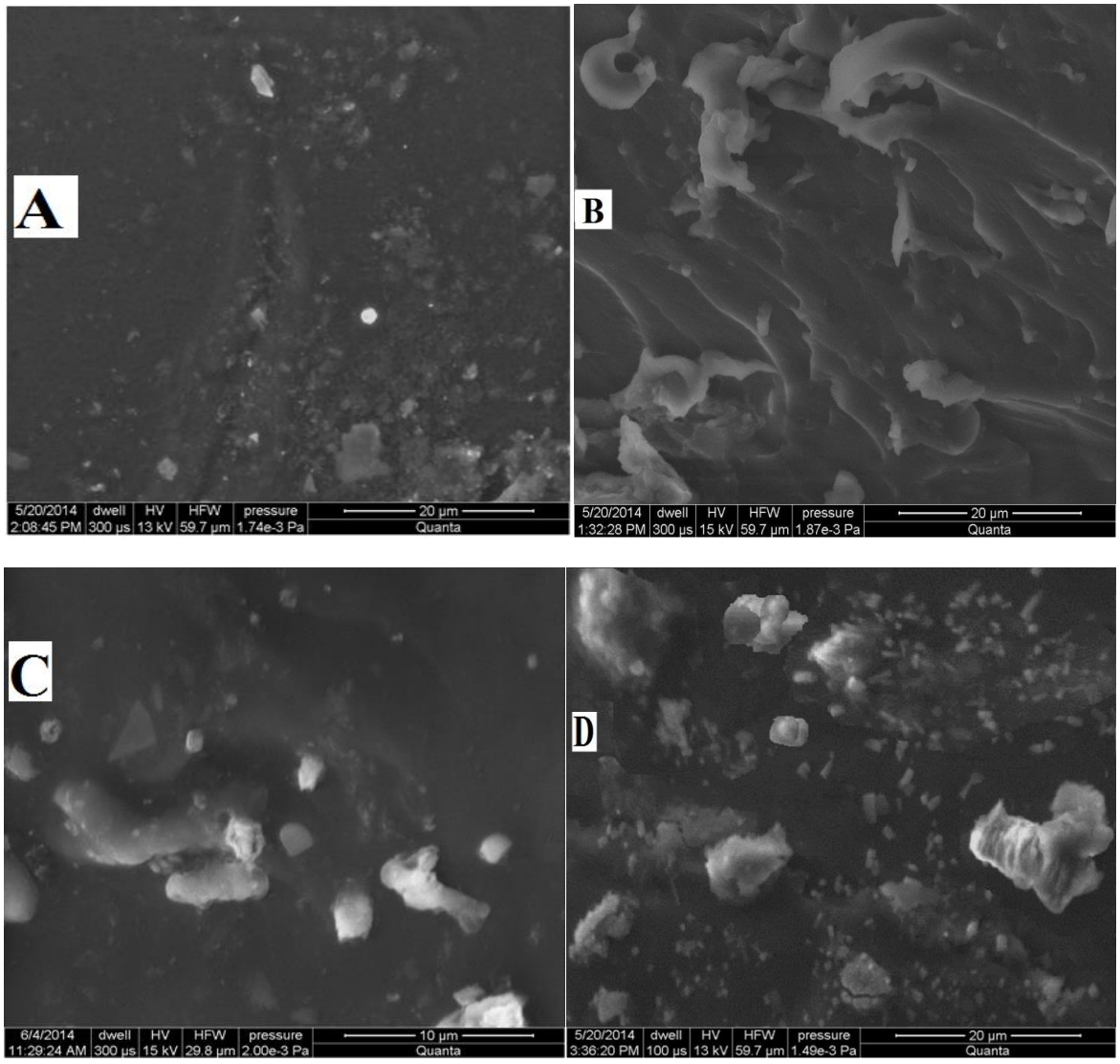

Figure 3. SEM Micrographs of Drug free (A and B) and Drug loaded (C and D) HPMC/PVA-co-acrylic acid Hydrogels prepared by Microwave Radiation and Conventional water bath method, with same ratios of Polymers (Hydroxypropyl-methylcellulose and Poly(vinyl alcohol) and acrylic acid.

Figure 3 (C and D) shows hydrogels loaded with captopril as model drug, which is entrapped and adhered on the surface of matrix. Higher quantity of drug could be loaded into the formulations produced by microwave radiations as it shown figure 3 (C and D) showing drug loaded R and F hydrogels, respectively.

From SEM images, it was observed that the application of microwave irradiation to prepare semi-IPN created the hydrogels more porous than the gels synthesized by using water bath

heating method. These observations were relevant to the work done by Zhao *et al.*,[72] who prepared thermo-sensitive poly (N-isopropylacrylamide) (PNIPAAm) hydrogels using microwave radiations in terms of morphological analysis.

3.4.3 XRD

The X-ray powder diffraction patterns of pure drug are compared with drug-loaded hydrogels. X-ray diffractograms of Captopril, drug free Hydrogels and drug-loaded Hydrogels are presented in figure 4. Figure 4 (B) and figure 4 (C) presents the diffraction pattern of F3 and R3 formulations, respectively. Figure 4 (D) and 4 (E) show the diffractograms of their respective drug loaded formulations.

The XRD scan of pure captopril had shown characteristic sharp and intense peaks between 0° and 50° (2θ) due to its crystalline nature (Figure 4 A), The appearance of a sharp peak at 2θ= 27.79° is the characteristic of captopril. The diffractograms of hydrogels not containing the drug was dense with only two marked peaks at nearly 2θ= 44° and 2θ= 49°, in both F3 and R3 formulations. This similarity was due to same constitutions of F3 and R3 hydrogels.

It was observed from the diffractograms in both formulations (R3 and F3) that the drug free (B and C) and hydrogels loaded with captopril (D and E) had a variation in diffraction pattern due to entrapment of drug. However, in drug loaded hydrogels, the peaks were of less intensity and diffraction pattern was dense in comparison to that of pure drug in figure (A). Therefore, crystallinity of captopril was decreased after its entrapment into polymeric networks, indicating the amorphous dispersion of drug into the hydrogels. The resultant evaluation on the basis of XRD analysis was in relevance with observations made by Manjanna *et al.*[281] In that research work, Aceclofenac Sodium was loaded into Polysaccharide hydrogel beads. Another related work was done by Giri *et al.*,[282] where diltiazem hydrochloride was loaded into cross-linked biodegradable IPN hydrogel beads of pectin and modified xanthan gum. They had also noted similar diffraction patterns of pure drug, drug free hydrogels and drug loaded hydrogels.

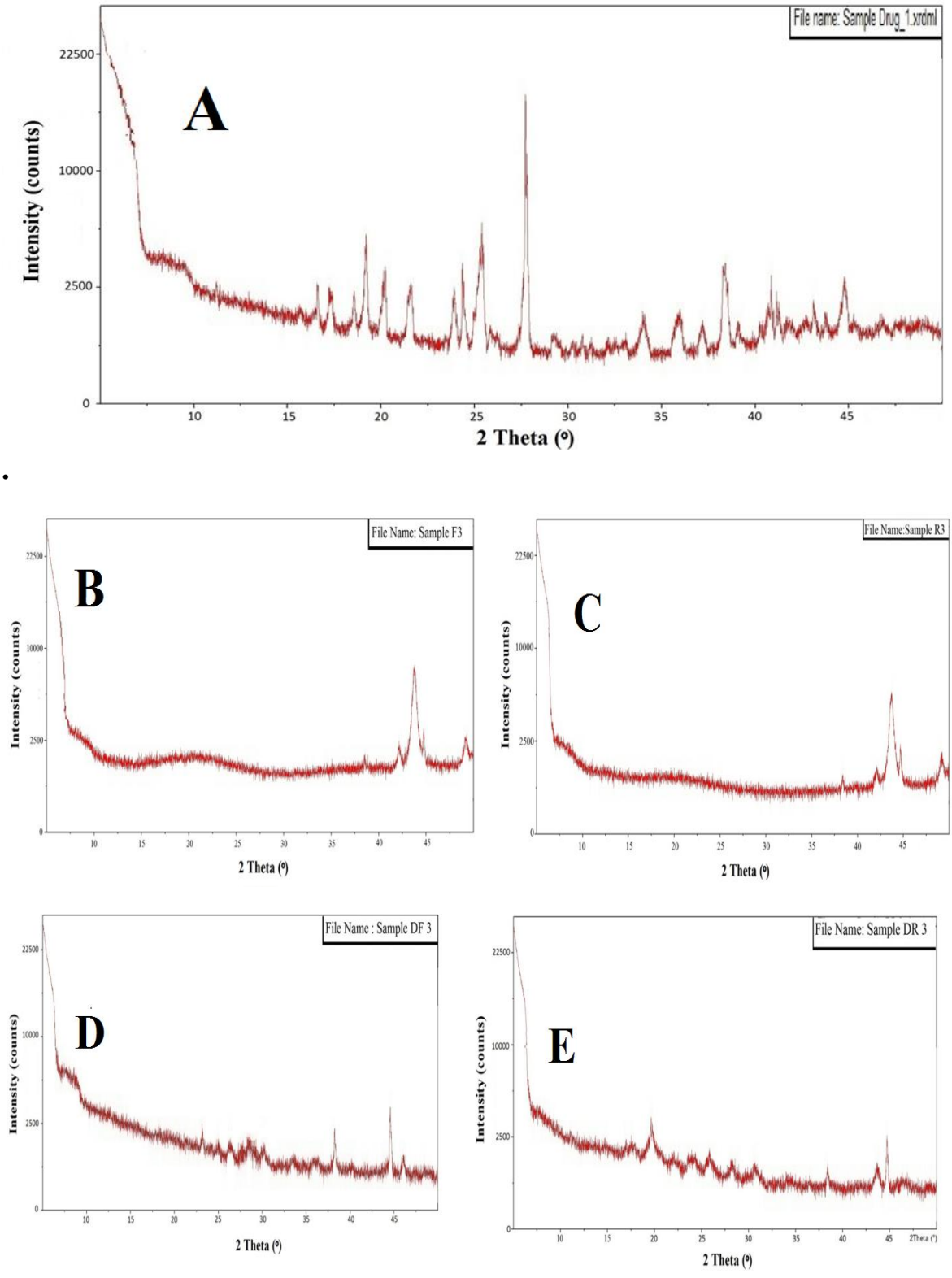

Figure 4. X- Ray Diffraction patterns of Captopril (A), Drug free Hydrogels (B and C) and Drug loaded Hydrogels (D and E)

3.4.4 Thermogravimetric analysis (TGA) and Differential scanning calorimetry (DSC)

The grafting of HPMC/PVA-co-AA semi interpenetrating network was supported by thermogravimetric analysis TGA. Figure 5 represents the comparative TGA patterns of individual components (HPMC, PVA and AA) as well as the crosllinked polymeric networks (hydrogel formulations R1 and F3).

TGA thermogram of HPMC shows decomposition at 265°C and continues upto 350 °C, till that period there was 78% weight loss due to degradation of the polymer. Similarly, the decomposition of Polyvinyl alcohol (PVA), starts at 220°C and continues to 320°C, showing 70% weight loss. The decomposition of pure acrylic acid starts at nearly 80°C and complete mass loss was observed at 189.50°C. However, TGA of the grafted product F3 is different having three stage of weight loss between 30°C and 500°C.

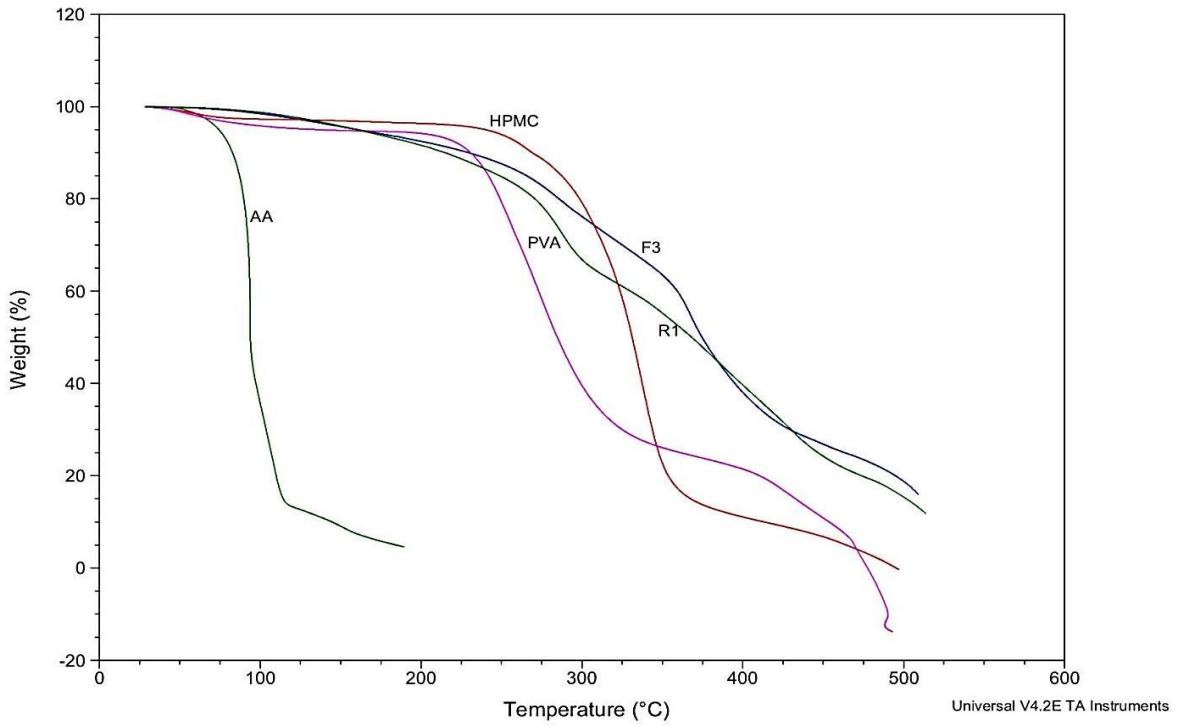

Figure 5. TGA thermograms of HPMC , PVA , AA (Acrylic acid), F3 hydrogel and R1 hydrogel

The first stage of weight loss starts at 80°C and continues up to 260°C, during which there was 15 % weightloss due to the loss of adsorbed and bound water. The second stage begins from 260°C to 360°C and this stage corresponds to 40 % weight loss. The third stage from

350°C to 500°C may contribute to the decomposition of different structure of the graft copolymer. At 490°C and 475°C there was complete weight loss of HPMC and PVA, respectively, whereas their copolymer had 80% weight loss at that temperature. These observations indicate the thermal stability of formed hydrogels in comparison to individual polymers and monomers (PVA, HPMC and acrylic acid). TGA thermograms determined in present work, indicate similarity to TGA patterns of Graft copolymer of chitosan with acrylic acid under microwave irradiation by Huacai *et al.*,[283] where thermal degradation was in three stages. Similar observations on basis of thermal analysis were also evaluated by Bao *et al.*[95] and Thakur *et al.*[284]

The DSC endothermic peaks of pure HPMC, PVA, AA and cross-linked polymeric network were in accordance with TGA thermal patterns. DSC is a well-known and established method often used as a qualitative technique for characterization of polymeric drug delivery systems. It determines the physicochemical changes in either enthalpy or heat capacity of a crystalline drug in the polymer matrix during the manufacturing process. The thermal behavior of pure Captopril, drug loaded R1 (DR1) and F3 (DF3) Hydrogels characterized by DSC are presented in figures 6a and 6b.

The thermogram of pure Captopril showed a sharp endothermic peak at 106°C followed by corresponding melting point. However, in DSC thermogram of Drug loaded samples DR1 and DF3, peaks were observed at 288.28°C and 265.29°C, respectively. The appearance of these peaks suggested that the drug loaded hydrogels showed an increase in the exothermic peak temperature as presented in figures 6 a and 6 b. The extra obvious peak of drug (106°C) was not observed in any type of the prepared hydrogels containing drug. The thermal stability of hydrogels was maintained after the incorporation of captopril. No marked change in thermal behavior of drug was indicated from the DSC thermogram of drug loaded hydrogels. It is indicated that captopril is uniformly dispersed into the polymeric network of HPMC, PVA and acrylic acid. This thermal evaluation of drug loaded hydrogels is in relevance to that determined by Manjanna *et al.*[281]

The TGA and DSC thermograms of both hydrogel formulations synthesized by different microwave radiations (R1) and conventional water bath (F3) were almost similar as could be clearly seen in figures 5 and 6. Hence, it means that the grafting of these polymers by both

methods increases the thermal stability of the polymers. These results are corresponding with the earlier findings of Zhao *et al.*[72]

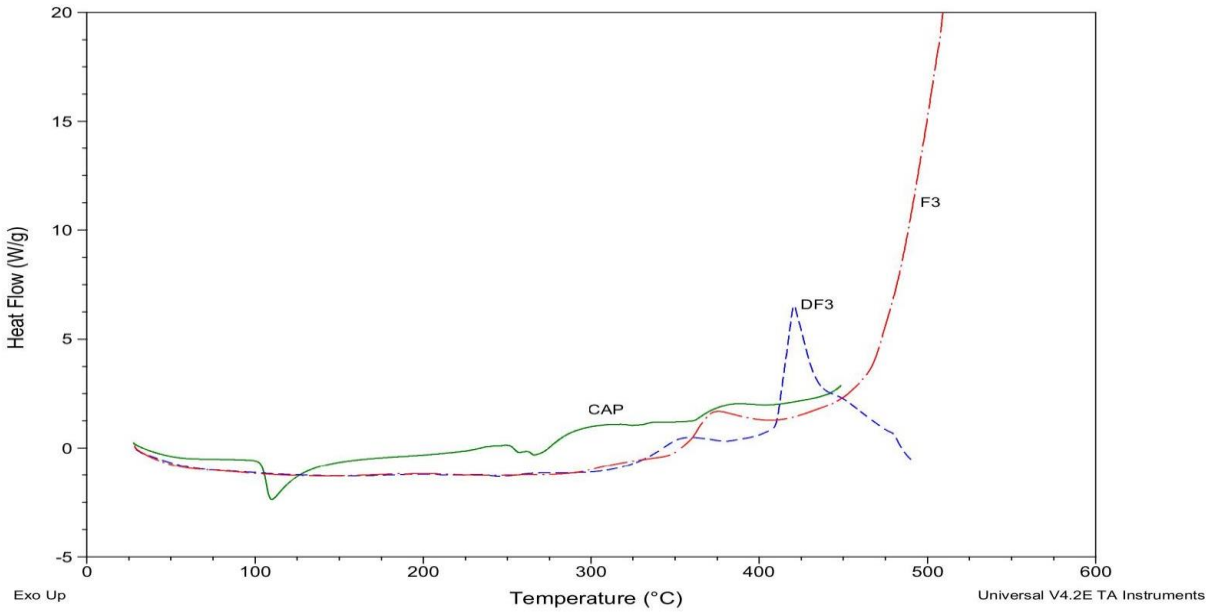

Figure 6a. DSC thermograms of captopril, drug free F3 hydrogel and drug loaded F3 hydrogel (DF3)

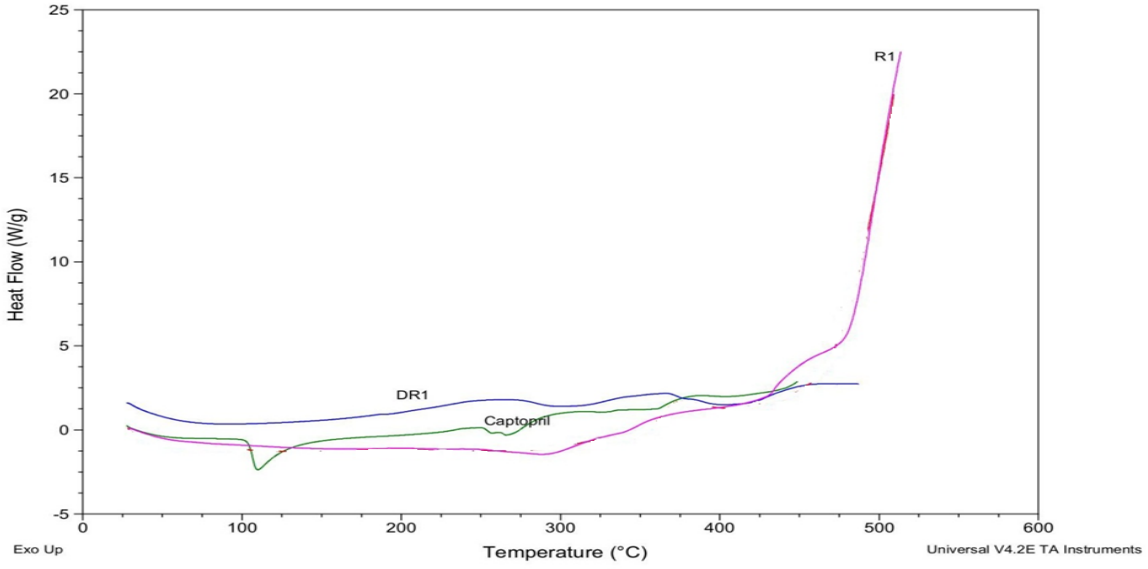

Figure 6b. DSC thermograms of captopril, drug free R1 hydrogel and drug loaded R1 hydrogel (DR1)

3.4.5 Swelling Study

The effect of using different proportions of HPMC and PVA on swelling capacity of hydrogels have been presented in figure 7 (a, b and c) at three levels of pH (1.2, 4.5 and 7.4), respectively. Similarly, the effect of crosslinking agent (MBA) on swelling at these pH values are presented in (d, e and f) in figure 7. Alteration in swelling ratio observed with different concentration of acrylic acid are shown in figure 7 (g). Higher swelling ratios were observed at higher pH values.

Effect of Polymer's proportions

The hydrogel P1 contains highest amount of HPMC among other hydrogel formulations containing same concentrations of acrylic acid and crosslinker. The swelling ratio is decreased by using higher amounts of PVA as it is lowest in P5 hydrogels with HPMC and PVA content in a ratio of 1:3. On the other hand, hydrogel P1 had HPMC and PVA content in a ratio of 3:1 (inverse of P5 content ratio). It indicated that HPMC has greater swelling capacity in comparison to PVA.

Effect of crosslinker concentration

It can be observed from the figure 7 (d, e and f) that increasing concentration of crosslinking agent leads to decrement in swelling power of hydrogels. This is because, it increases the grafting of polymers and monomer crosslinking. Due to higher concentrations of MBA, the crosslink density increases that makes the hydrogels lesser spaces for incorporation of aqueous solutions, which results in lower swelling ratios. These results were similar to earliaer observations of Huacai *et al.*,[283] who determined an increment in percent grafting of chitosan-g- acrylic acid hydrogels with increasing amount of MBA that decreased the swelling behavior.

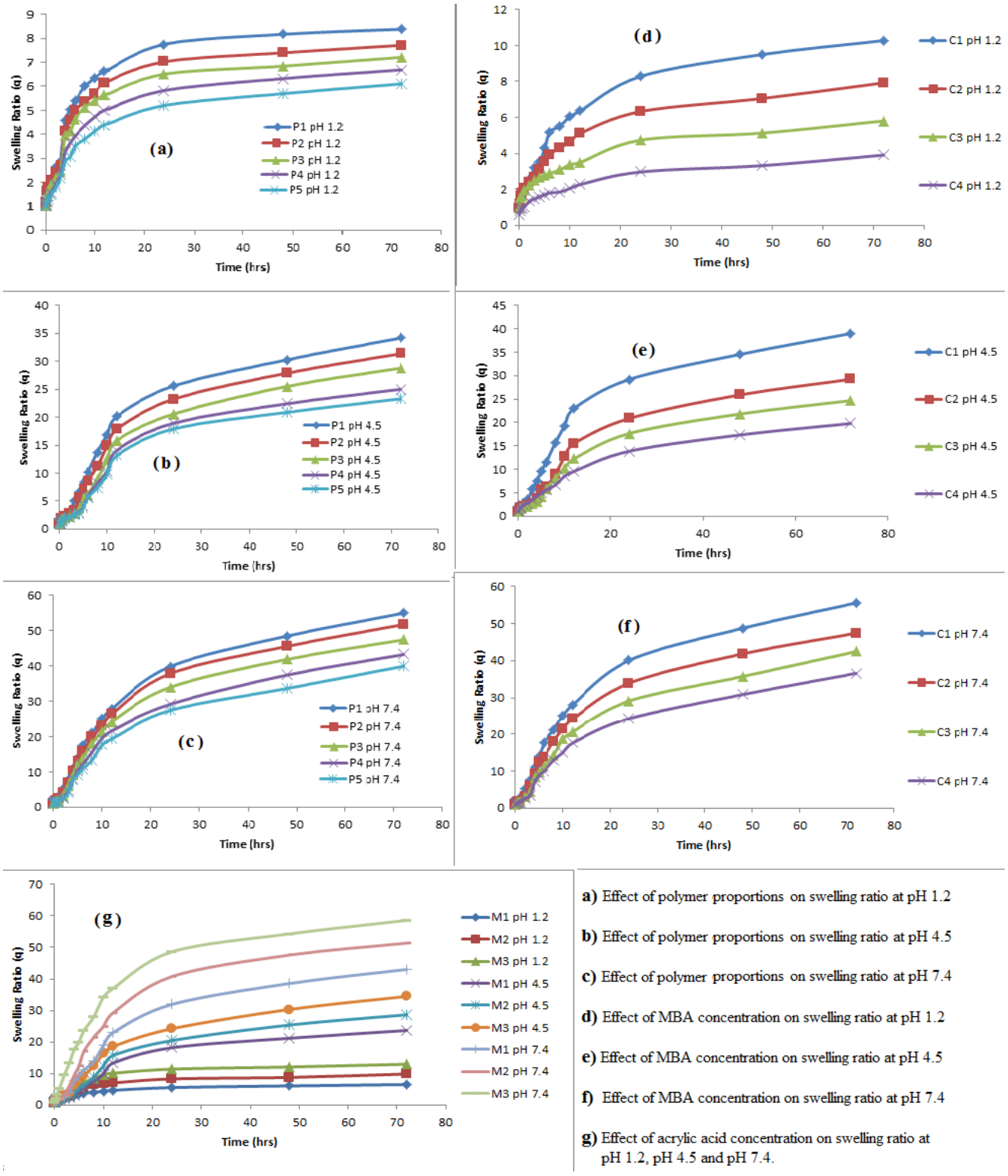

Figure 7. Effect of polymer's proportion, MBA and acrylic acid concentrations on swelling ratio

Effect of acrylic acid concentration

Figure 7(g) shows that by increasing the concentration of acrylic acid create an enhancement in swelling ratio. Hydrogel formulations M1, M2 and M3 have the same amounts of components except acrylic acid. M1 contains acrylic acid 20% w/w exhibit higher swelling tendency in comparison to M2 and M3 containing lower amounts of monomer. The noted results were according to swelling pattern observed by Ranjha *et al.*[281] where, swelling tendency of pectin/acrylic acid hydrogels was higher in formulations containing higher amounts of acrylic acid.

3.4.6 Comparative Swelling of F and R hydrogels

Swelling ratios of the synthesized gels were measured gravimetrically at the pH 1.2, 4.5 and 7.4 in phosphate buffer. The swelling of hydrogels is dependent upon the presence of hydrophilic groups (ionizable functional groups). PVA and HPMC contain high hydroxyl groups that make this polymer highly interactive with water. The carboxylic groups of acrylic acid were mainly responsible for swelling tendency in hydrogels. When the pH is increased, the COOH was ionized and deprotonated to a negatively charged COO^{--}. As a result, the electrostatic repulsion causes the swelling of hydrogel and greater expansion of the network thus gives a high swelling ratio. The swelling ratios of hydrogels noted were similar to swelling measurements performed by Gemeinhart *et al.*[286] for poly (acrylamide-co-acrylic acid) super porous hydrogels. The swelling study was also relevant to work done by Shah *et al.*[287] Therefore, the carboxylic groups associated with acrylic acid made the coploymeric system a pH responsive that could be observed from swelling characteristics. Effect of polymer concentration and radiation dose on swelling ratio at various pH were studied to evaluate the swelling with respect to time.

As shown in figures 8 and 9, hydrogels containing higher amount of polymer show lesser comparative swelling ratios. R1 and F1 comprising lower amount of polymer exhibited higher swelling behaviour as compared to the other formulations. As the quantity of polymer increases in the hydrogels, the value of their swelling ratio decreases. Generally, a more porous matrix provides more space to accommodate larger quantities of water.[288] As shown in figures 3 (SEM images), the R hydrogels had more porous structure than the F hydrogels, therefore, it would have higher water uptake. As a result, the hydrogels prepared

by microwave radiation method show better swelling characteristics in comparison to conventional methods. Thus, microwave radiations develop uniform crosslinking and a more porous polymeric system than simple water bath heating method. It could be seen from comparative swelling ratios of R4 and F4 formulations in figure 10, which depicts higher swelling of R4 formulation at pH 1.2 and pH 7.4.

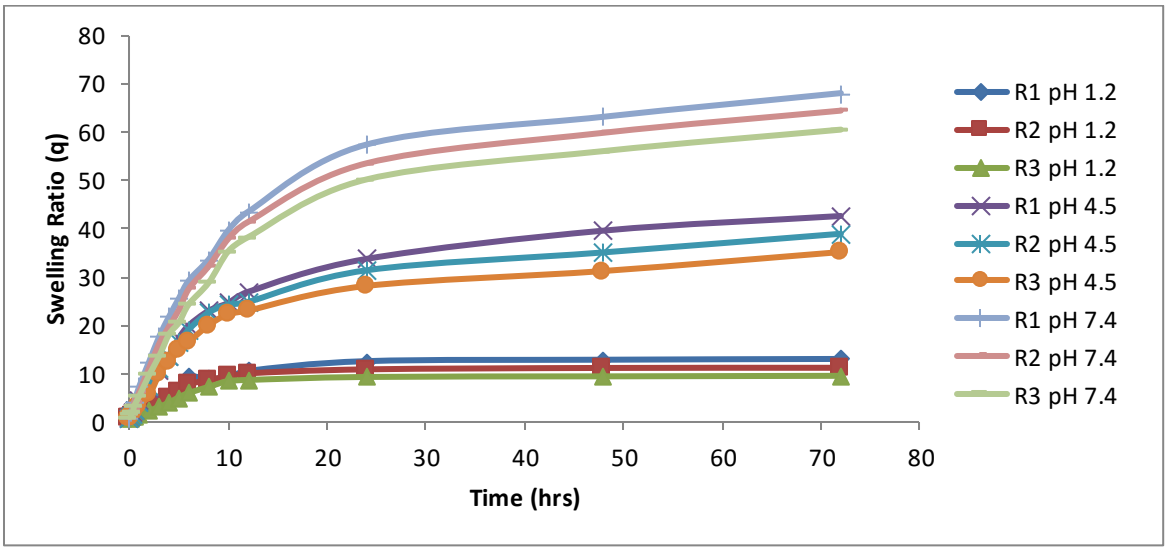

Figure 8.Swelling ratios of the Semi IPN hydrogels synthesized by Microwave Radiations

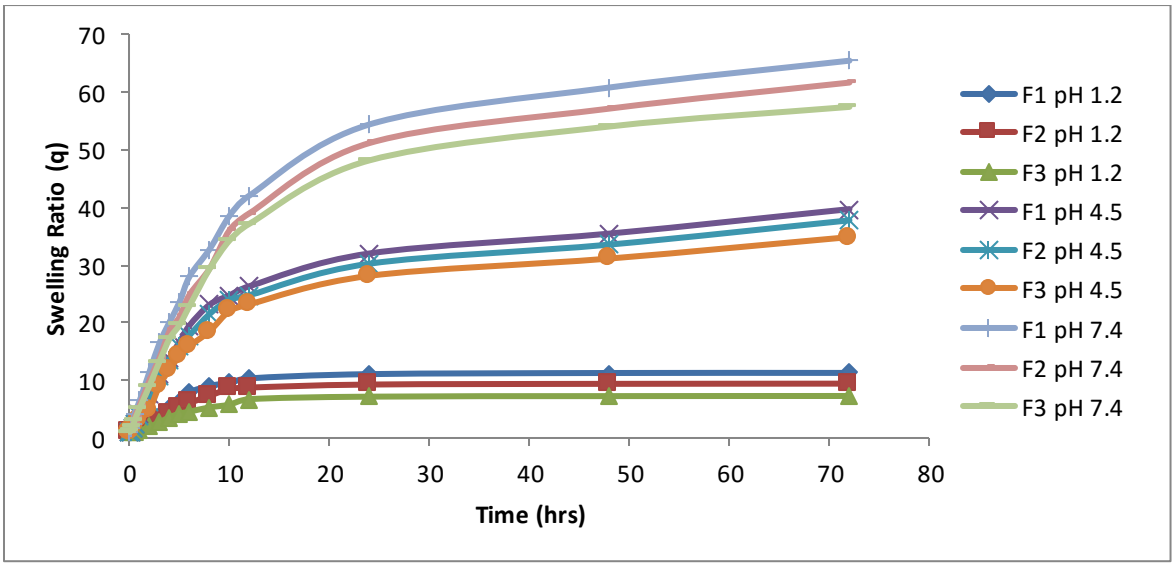

Figure 9. Swelling ratios of the Semi IPN hydrogels synthesized by Conventional Water bath Method

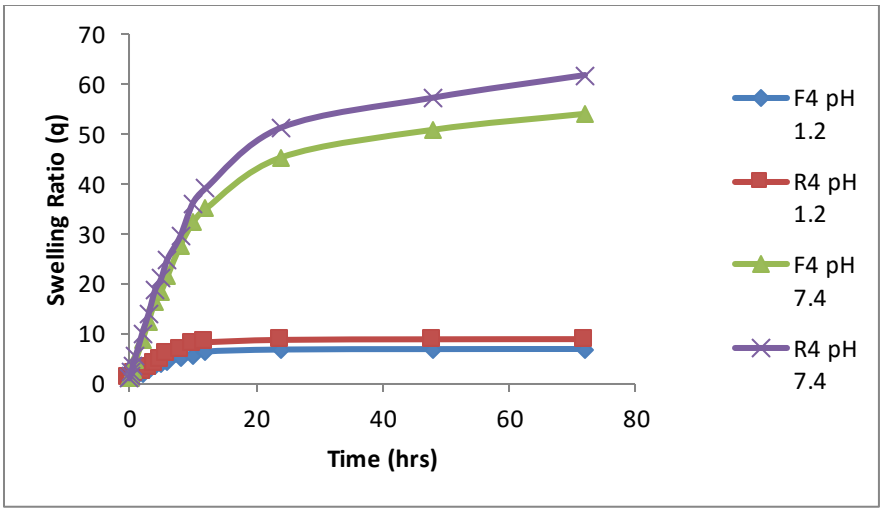

Figure 10. Comparative Swelling ratios of the hydrogels (R4 and F4)

Hydrogel synthesized at higher dose always have lower swelling ratio probably due to the higher crosslink density which resist the swelling of matrix. The comparative swelling ratios of hydrogels treated finally at 300W for 2.5, 5, 7 and 10 minutes are presented in figure 11.

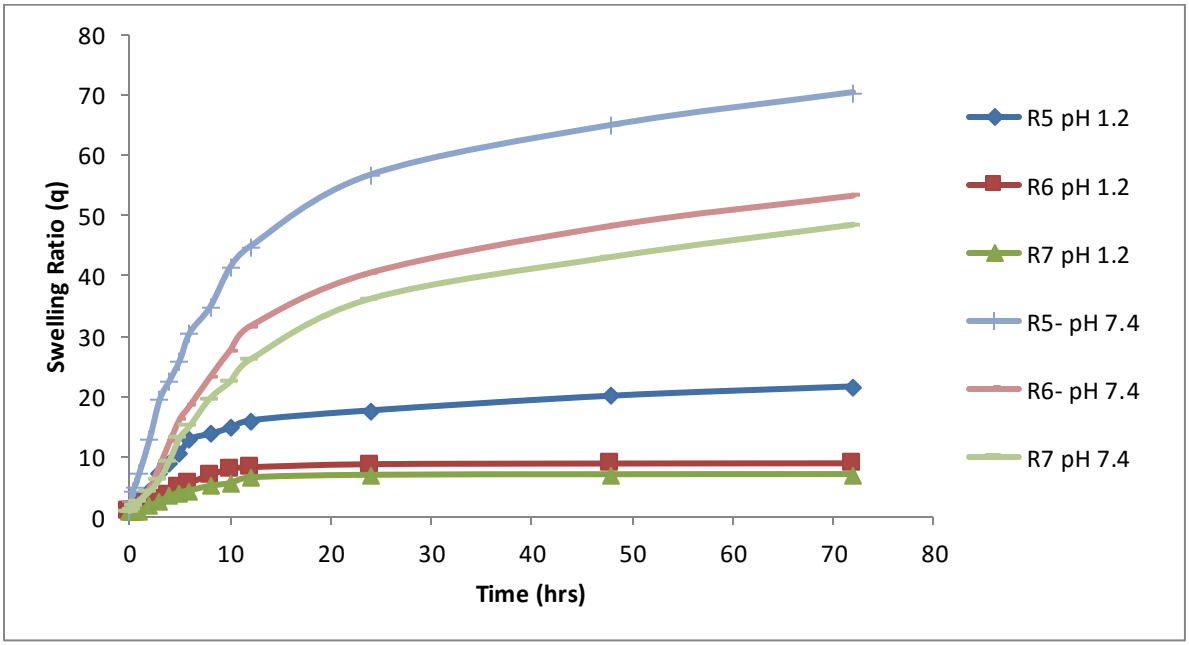

Figure 11. Comparative Swelling ratios of the hydrogels prepared at different radiation doses

It is also observed that hydrogels prepared at 300W for 5 min (R1, R3 and R4) had higher swelling ratio as compared to the hydrogel with final exposure of 7.5 min (R6) and 10 min (R7) but swells less than R5 treated for 2.5 minutes. It had been already determined by Wan

et al.,[274] that increasing the exposure time of microwave radiation increases the grafting of polymers and ultimately higher crosslinking in polymeric network. This may be concluded from the lower crosslink density of hydrogel which formed a porous structure and thus allowed the water molecules to diffuse easier, hence faster response time.

3.4.7 Gel Fraction

The percent gel fraction was determined for formulations with different concentration of polymers (HPMC and PVA), monomer (acrylic acid) and crosslinking agent (MBA). The values of percent gel fraction are presented in table 6.

Table 6. Percent gel fraction of formulations with different amounts of components

Formulation code	W_i (g)	W_e (g)	% gel fraction
F1	0.321	0.277	86.3
F3	0.328	0.289	88.1
F5	0.326	0.295	90.4
M3	0.313	0.269	85.9
M2	0.318	0.284	89.3
M1	0.324	0.301	92.9
C2	0.311	0.276	88.7
C3	0.315	0.286	90.7
C4	0.322	0.302	93.7

The amount of polymer, monomer and crosslinker are among the main factors affecting the gel fraction of hydrogels. It could be observed from the figures 11, 12 and 13 depicting the effect of concentration of polymer, monomer and crosslinker on per cent gel fraction. Increasing the concentration of these components increases the value of %GF.

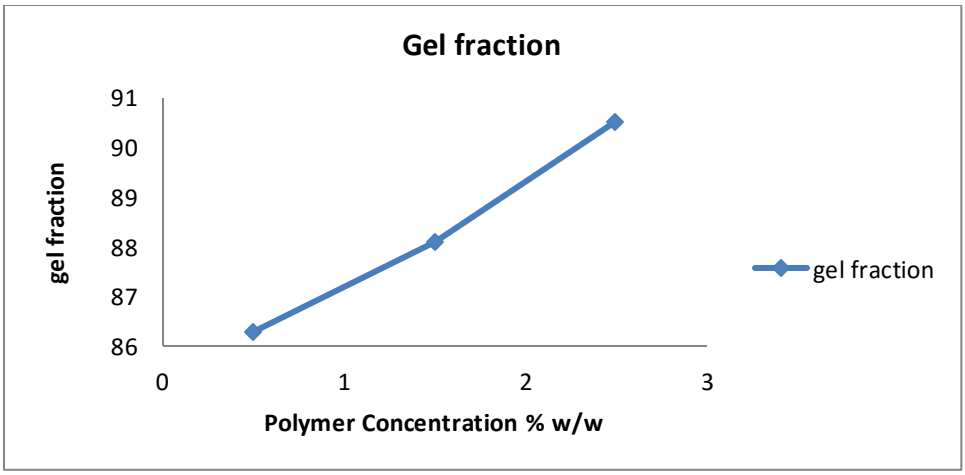

Figure 12.Effect of polymer concentration on %GF.

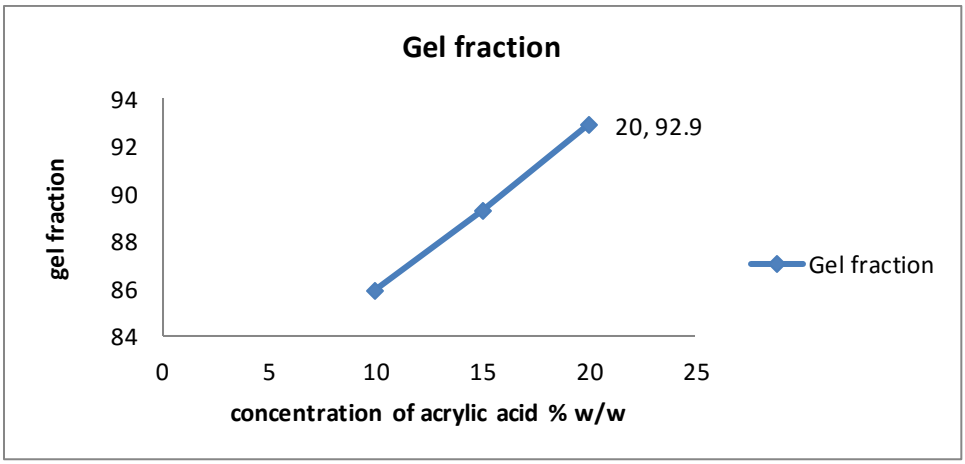

Figure 13.Effect of monomer concentration on %GF.

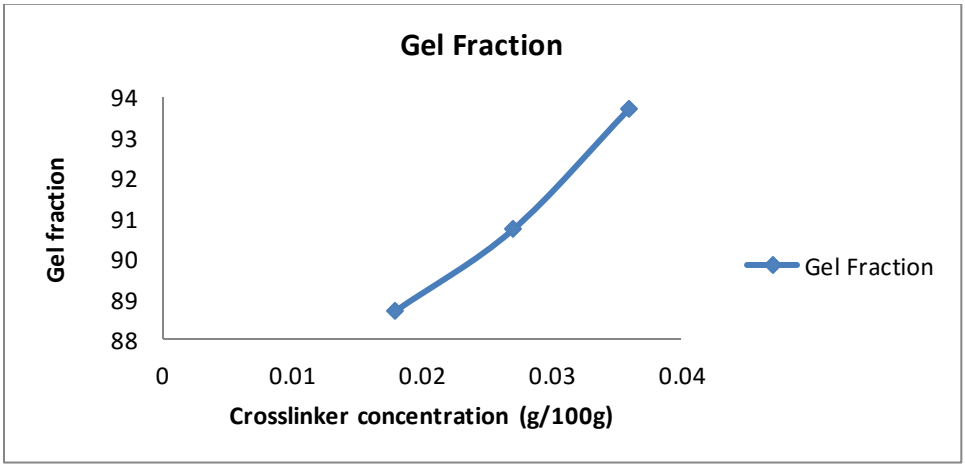

Figure 14.Effect of crosslinker concentration on %GF.

Figure 11 indicates that by increasing the concentration of polymer (HPMC and PVA, 3:1) from 0.5% to 2.5%, an increment in value of %GF has been observed from 86.3% to 90.4%, respectively. In comparison to polymer concentration, the monomer quantity used has more influence on gel fraction due to its relatively higher concentrations used in hydrogel formation. Figure 12 shows that by increasing the amount of acrylic acid from 10% to 20%, percent gel fraction was increased from 85.9% to 92.9%. Moreover, crosslinker's concentration has similar effects on percent gel fraction because of enhancing crosslinking interaction of acrylic acid with polyvinyl alcohol, which are then interacted with hydroxypropyl methyl cellulose. Yoshii *et al.*[289] and Roberta *et al.*[290] had used the same technique for determination of percent gel fraction and obtained similar results of higher %G with increasing concentration of constituents.

3.4.8 Drug loading and release studies

The hydrogel discs exhibiting greater swelling accommodated higher amounts of drug. The amount of captopril loaded in hydrogel formulation and percent drug release at acidic pH and higher pH has been presented in table 7. The dissolution study of captopril was performed at pH 1.2 and pH 7.4 as shown in figure 15 and figure 16. The drug release at both pH (1.2 and 7.4) was observed for a period of 24 hours, where USP phosphate buffer was used as dissolution medium. Drug release measured is directly related to swelling studies where relatively more amount of drug was loaded and ultimately released in formulations exhibiting more swelling tendency.

Table 7. Amount of Captopril loaded and percentage of drug release at pH 2 and pH 7.4

Formulation code	Amount of captopril loaded (mg) per 0.3 gram of dry hydrogel discs	% release of captopril (for 24 hour period)	
		pH 1.2	pH 7.4
F1	98.13	31.54	82.15
F2	95.08	29.49	80.06
F3	89.58	26.88	78.57
F4	83.50	24.72	75.79
R1	104.5	35.65	87.92
R2	97.81	32.84	85.01
R3	92.28	28.96	83.56
R4	86.8	26.45	78.54

It could be clearly observed from table 7, that hydrogels possessing higher swelling ratios had entrapped more quantity of drug. R1 and F1 had same ratios of polymers (HPMC and PVA), acrylic acid and crosslinking agent, but they were prepared using different techniques. The captopril loaded in R1 formulation was 104.5 mg in 0.3 gram dry hydrogel disc, whereas it was 98.13 mg in F1 formulation, hence, relatively higher amount of captopril was loaded. This difference could also be noticed in other formulations prepared using microwave radiations (R2, R3 and R4) and conventional water bath (F2, F3 and F4).

These variations are may be due to uniformity in porous structure and presence of larger voids in R hydrogel formulations, leading to higher and more uniform dispersion of drug in polymeric network. Moreover, factors such as polymer concentration, monomer concentration and other factors affecting swelling behavior of hydrogels directly affect drug loading and release.

The cumulative percent drug release from hydrogel formulation F2 and R2 have been presented in figure 15 and figure 16. The drug release pattern at both lower pH 1.2 and higher pH 7.4, could be seen from the figures 15 and 16.

A similarity in release pattern was observed, as both formulations were releasing more drugs at higher pH values. At pH 1.2, the cumulative percent drug release was 29.49% in F2 and 32.84% in R2 hydrogels, while at pH 7.4, drug release were 80.06% and 85.01% for F2 and R2 formulations, respectively. These values of percent drug release are also mentioned in table 7. Therefore, the drug release is dependent upon the pH sensitivity imparted due to presence of acrylic acid in hydrogels. The cumulative percent drug release is in correspondence to the results obtained by swelling study.

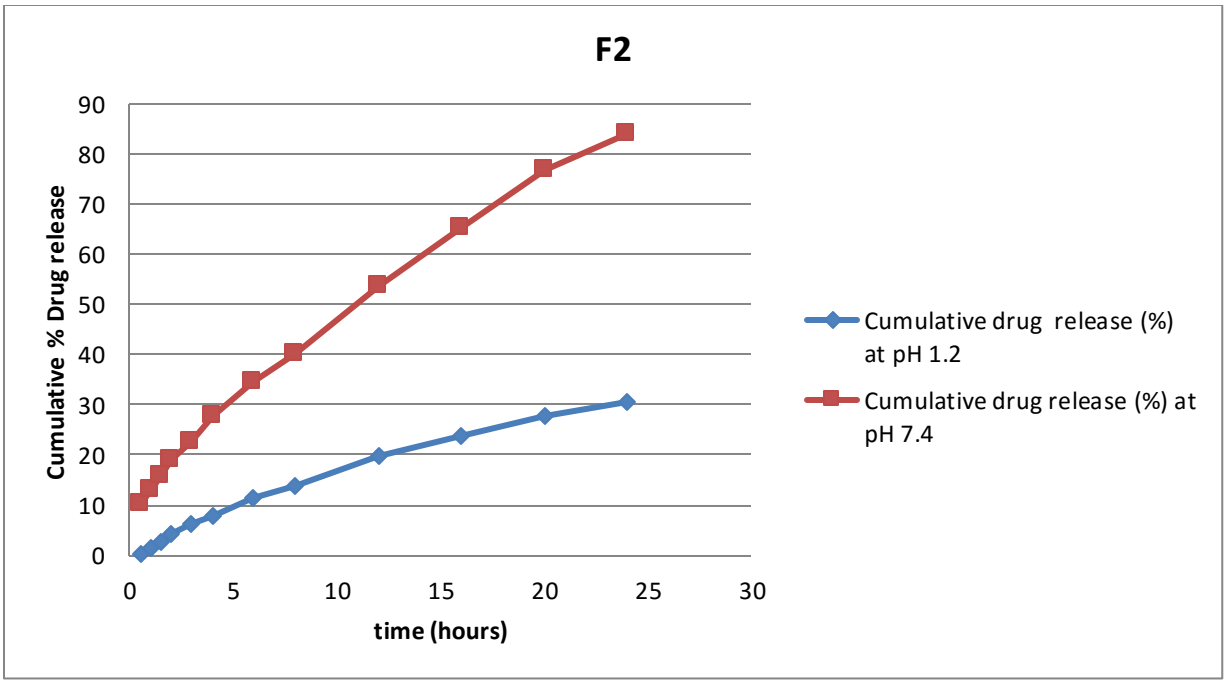

Figure 15. Captopril released upto 24 h from HPMC/PVA-co-AA hydrogel (F2) in solutions with pH 1.2 and pH 7.4

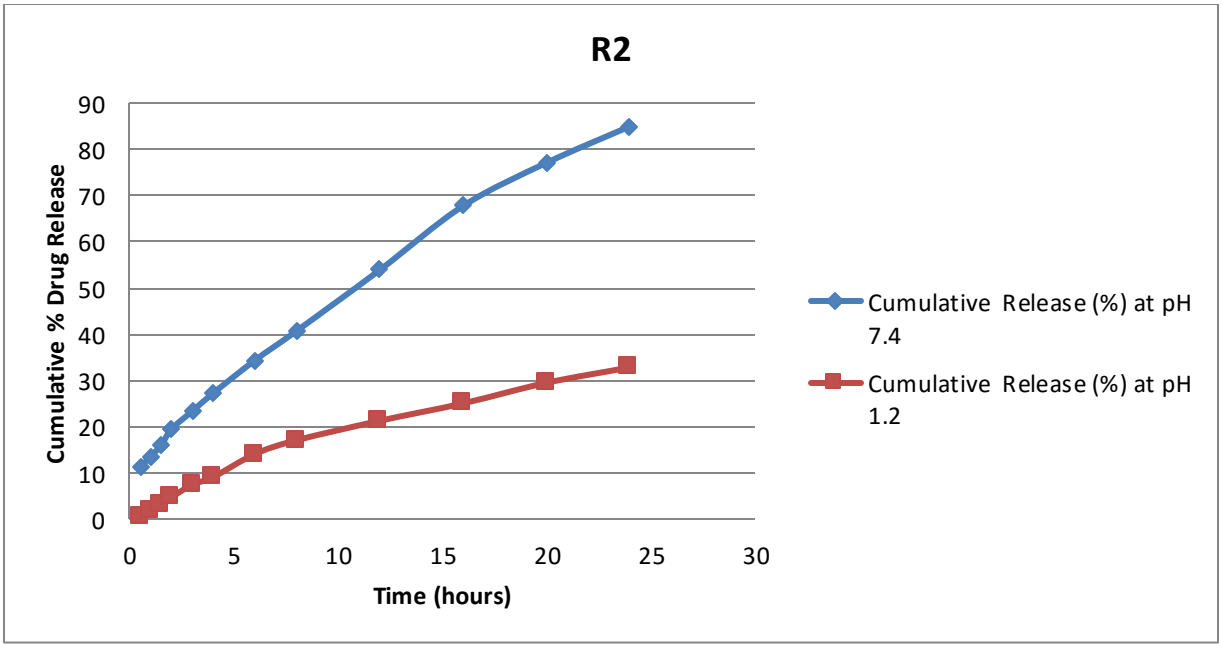

Figure 16. Captopril released upto 24 h from HPMC/PVA-co-AA hydrogel (R2) in solutions with pH 1.2 and pH 7.4

The drug release was evaluated by the application of zero order kinetics, first order kinetics, Higuchi model, Korsmayer-Peppas model and Weibull model. The values of release coefficient R^2 and K calculated by these kinetic models are presented in table 8. The captopril released from F and R hydrogel formulations were best fitted into the kinetic model having value of R^2 close to 1.

Table 8: Determination of coefficient (R^2), K and release exponent of various drug release kinetic models

Sample code	pH	Zero order kinetics		First order kinetics		Higuchi model		Korsmeyer Peppas model			Weibull model
		R^2	K	R^2	K	R^2	K	R^2	K	n	R^2
F1	1.2	0.9719	1.84	0.7215	0.165	0.9969	4.83	0.9913	3.23	0.703	0.7012
	7.4	0.9874	3.96	0.5248	0.263	0.9876	14.81	0.9967	13.57	0.5488	0.8955
F2	1.2	0.9767	1.69	0.702	0.156	0.9963	4.33	0.996	3.54	0.598	0.669
	7.4	0.989	3.94	0.5274	0.261	0.9901	14.586	0.9979	12.022	0.609	0.8905
F3	1.2	0.9811	1.45	0.7674	0.134	0.9917	3.54	0.9968	3.37	0.5221	0.7395
	7.4	0.9843	3.76	0.539	0.2557	0.9902	13.793	0.9971	13.88	0.496	0.8835
F4	1.2	0.9736	1.42	0.7598	0.158	0.9955	3.06	0.9864	1.952	0.7042	0.7775
	7.4	0.99	3.59	0.6252	0.238	0.9844	11.56	0.9966	9.93	0.579	0.8774
R1	1.2	0.9815	1.83	0.7362	0.169	0.9928	5.12	0.9954	4.2945	0.587	0.7544
	7.4	0.9812	4.179	0.5054	0.2668	0.9921	15.92	0.9968	15.58	0.5118	0.8804
R2	1.2	0.9632	1.86	0.7083	0.1674	0.9975	4.93	0.9881	3.59	0.659	0.6939
	7.4	0.9896	3.85	0.5239	0.262	0.9882	14.814	0.9973	13.4	0.556	0.8983
R3	1.2	0.9805	1.61	0.7532	0.1513	0.9929	4.03	0.9963	3.54	0.56	0.5894
	7.4	0.9817	4.02	0.5267	0.26	0.9912	14.472	0.9956	15.02	0.479	0.8771
R4	1.2	0.9761	1.48	0.7024	0.135	0.9949	3.52	0.9965	3.33	0.524	0.7314
	7.4	0.9877	3.64	0.5642	0.25	0.9876	13.194	0.9983	12.6	0.525	0.8896

As shown in table 8, the value of drug release coefficient (R^2) calculated by zero order was ranging from 0.97 to 0.99. Similarly, the values of R^2 calculated by Higuchi model and Korsmeyer Peppas model were in that range. Hence, it could be observed that all captopril loaded hydrogel formulations prepared were following zero order kinetics, Higuchi model and Korsmeyer Peppas model in terms of drug release. The mechanism of drug release was indicated by the values of n i.e. release exponent. By fitting of recorded data to Peppas model, it was investigated that approximately all hydrogel formulations in spite of having different concentrations of polymer and monomers were following non- Fickian mechanism of drug release as presented in Table 8. The value of n in all cases were more than 0.45 but lesser than 1.

Conclusions

The above discussion concludes that use of microwave irradiation is more efficient technique for hydrogel synthesis for the components used in this study. It can remarkably shorten the reaction time required for polymer crosslinking from several hours to few minutes in comparison to conventional hydrothermal method. The polymeric network HPMC-g-PVA-co-poly (acrylic acid) was prepared successfully by induction of microwave radiations. The hydrogels formed by using this method had comparatively higher swelling tendency and drug loading. Therefore, microwave assisted hydrogel synthesis developed a promising drug carrier that could be effectively utilized for delivering captopril for longer time period in a controlled manner. It could be a suitable candidate to prove its worth in the treatment of hypertension and certain other heart disorders.

Chapter no. 4

Synthesis and *in-vitro* characterization of Hydroxy propyl methylcellulose-g-poly(acrylic acid-co-2-Acrylamido-2-methylpropane sulfonic acid) polymeric network for controlled release of captopril

Summary

Background of the Study

A super-absorbent hydrogel was developed by crosslinking of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) and acrylic acid with hydroxypropyl methylcellulose(HPMC) for controlled release drug delivery of captopril, a well-known antihypertensive drug.

Methods

Acrylic acid and AMPS were polymerized and crosslinked with HPMC by free radical polymerization, a widely used chemical crosslinking method. N,N'-methylenebisacrylamide (MBA) and potassium persulfate (KPS) were added as cross-linker and initiator, respectively. The hydrogel formulation was loaded with captopril. The concentration of captopril was monitored at 205 nm using UV spectrophotometer. Equilibrium swelling ratio was determined at pH 2, 4.5 and 7.4 to evaluate the pH- responsiveness of the formed hydrogel. The hydrogels were evaluated by FTIR, SEM, XRD, and thermal analysis (DSC and TGA).

Results

The formation of new copolymeric network was determined by FTIR, XRD, TGA and DSC analysis. The hydrogel formulations with acrylic acid and AMPS ratio of 4:1 and lower amounts of crosslinker had shown maximum swelling. Moreover, higher release rate of captopril was observed at pH 7.4 than at pH 2, because of more swelling capacity of copolymer with increasing pH of the aqueous medium.

Conclusions

The present research work confirms the development of a stable hydrogel comprising of HPMC with acrylic acid and AMPS. The prepared hydrogels exhibited pH- sensitive behavior. This superabsorbent composite prepared could be a successful drug carrier for treating hypertension.

Keywords: Composite, Superabsorbent, Polymerization, Acrylic acid, 2-Acrylamido-2-methyl-1-propanesulfonic acid, Hydroxypropylmethylcellulose, Initiator, Cross linker, Hydrogel.

4.1 Introduction

Drug delivery systems have been known for enhancing therapeutic efficacy, minimizing side effects, and improving patient compliance. Among drug delivery systems, hydrogels have attracted the interest of biomaterial scientists due to their hydrophilic character and biocompatibility.[291] Due to their softness and pliable nature, they have tendency to absorb higher amounts of water and physiological solutions, these absorbed aqueous solutions are capable of retaining water even if subjected to pressure. They have numerous applications as drug carriers, water-absorbents and food additives.[292]

Superabsorbent hydrogels have an ability to absorb water from 10% to thousands times of their dry weight.[293] Due to special characteristics, these materials have gained attention in the fields of agriculture, waste water treatment,[294-296] pharmaceutical and biomedical[297,298] and biotechnology.[299,300] Polysaccharide-based hydrogels are currently attracting much interest fortheir unique properties, which are their better biocompatibility, biodegradability, renewability, and nontoxicity. Various polysaccharides, such as starch,[301] chitosan,[302] carrageenan,[303] alginate,[304] cellulose and cellulose derivatives[305–307] have been used for superabsorbent hydrogel formulation.

In this work, a super-absorbent hydrogel was synthesized by copolymerization of two monomers (acrylic acid and AMPS) and their crosslinking with HPMC using potassium persulfate to initiate the reaction and MBA as crosslinking agent. Hydroxypropylmethylcellulose (HPMC), the cellulose derivative, is a semisynthetic, inert, and viscoelastic polymer found in a variety of commercial products. Depending on the grade, HPMC is widely utilized in oral solid dosage forms (tablets and capsules) as well as an ophthalmic lubricant.[308,309] HPMC comprises of repeated units of glucose, linked with one another by 1 4-glycosidic bonding, while the polymer chains are attached together by hydrogen bonding.[310]

Acrylic acid (AA) has been extensively used as monomer in hydrogel synthesis due to relatively economical advantage, crosslinking ability as well as rapid polymerization by various formulation techniques. It possesses a pH and electrically responsive behavior due to ionic repulsion between anionic charged carboxylate groups. It polymerizes to polyacrylic

acid (PAA) an established vehicle in controlled release drug delivery.[277,311] 2-Acrylamido-2-methyl-1-propanesulfonic acid (AMPS) it has attracted an attention in hydrogel formation possess due to presence of sulfonate groups. Strongly ionizable sulfonate groups increase the hydrophilicity and ultimately swelling capability of hydrogels. The polymeric network comprising of AMPS have ability to swell at all pH ranges, therefore it does not impart a pH-sensitive behavior to its hydrogel formulation. As stated in literature, it swells rapidly in acidic medium and relatively slower at pH higher than 5. AMPS contain both nonionic and anionic groups; whereas, AA is an anionic monomer.[312] Due to these characteristics acrylic acid was combined with AMPS, to release the drug loaded in a controlled manner. The hydrogels prepared were observed for their swelling behavior different pH (2, 4.5 and 7.4). They were loaded with captopril and its release in was studied by dissolution process at pH 2 and pH 7.4. Moreover, FTIR, SEM, XRD, DSC and TGA analysis were performed for *in-vitro* characterization of super-absorbent composite.

4.2 MATERIALS AND METHODS

4.2.1 Chemicals

Hydroxypropylmethylcellulose (2600−5600 cps, Sigma Aldrich, Netherlands), acrylic acid (Sigma Aldrich-Netherlands), AMPS, (99%, Aldrich-product of Germany) N,N-Methylene-bis-acrylamide (98%, Fluka-Switzerland), potassium persulphate (Analar, BDH-England), and potassium dihydrogen phosphate (Merck, Germany) were purchased through local commercial sources. Distilled water from laboratory and solvents of analytical grades were used.

4.2.2 Preparation of hydrogel

The hydrogel was prepared by free radical polymerization, where the polymer (HPMC) in varying quantities was added in distilled water and stirred at 80ºC for 1 h. Then the HPMC solution subjected to nitrogen purging for about 30 min and potassium persulfate (0.5% W/W) was added to initiate the reaction by generating free radicals. After that the reactants was cooled down to 30ºC and MBA as cross-linking agent dissolved in acrylic acid (AA) was added under magnetic stirring. Then, AMPS previously dissolved in small quantity of water was added to above mixture and final volume was adjusted by addition of deionized distilled

water. After that, the above mixture was poured in test tubes and heated in water bath at 50°C, 55°C, 65°C and 75°C for 30 minutes, 1 hour, 2 hour and 3 hour, respectively. Then, the glass tubes were cooled to 25°C and hydrogels were taken out and cut in the form of discs of nearly 8mm long. They were then thoroughly treated with ethanol and distilled water mixture (50:50) for removing catalysts and uncross-linked monomer till the pH of solutions after washing becomes nearly same as before being used. After washing process, the hydrogel discs were air dried for overnight and then transferred to oven at 45°C for 4 to 5 days until they attain a constant weight. The whole crosslinking reaction initiated by potassium persulfate (KPS), involving interaction among polymer and monomers are presented in figure 1. Table 1 shows hydrogels prepared using different concentration of components.

Table 1. Hydrogels formulations using different amounts of HPMC, AMPS and MBA

Formulation code	Polymer (HPMC) g/100g	Monomers, g/100g			Crosslinking agent, mol % of each monomer's concentration
		AA	AMPS	AA/AMPS ratio	
S1	1.0	15	2. 5	6:1	0.6
S2	1.0	15	3	5:1	0.6
S3	1.0	15	3.75	4:1	0.6
S4	1.0	15	5	3:1	0.6
S5	1.0	15	7.5	2:1	0.6
S6	1.5	15	3.75	4:1	0.6
S7	2.0	15	3.75	4:1	0.6
S8	1.0	15	3.75	4:1	0.8
S9	1.0	15	3.75	4:1	1.0
S10	1.0	15	3.75	4:1	0.4

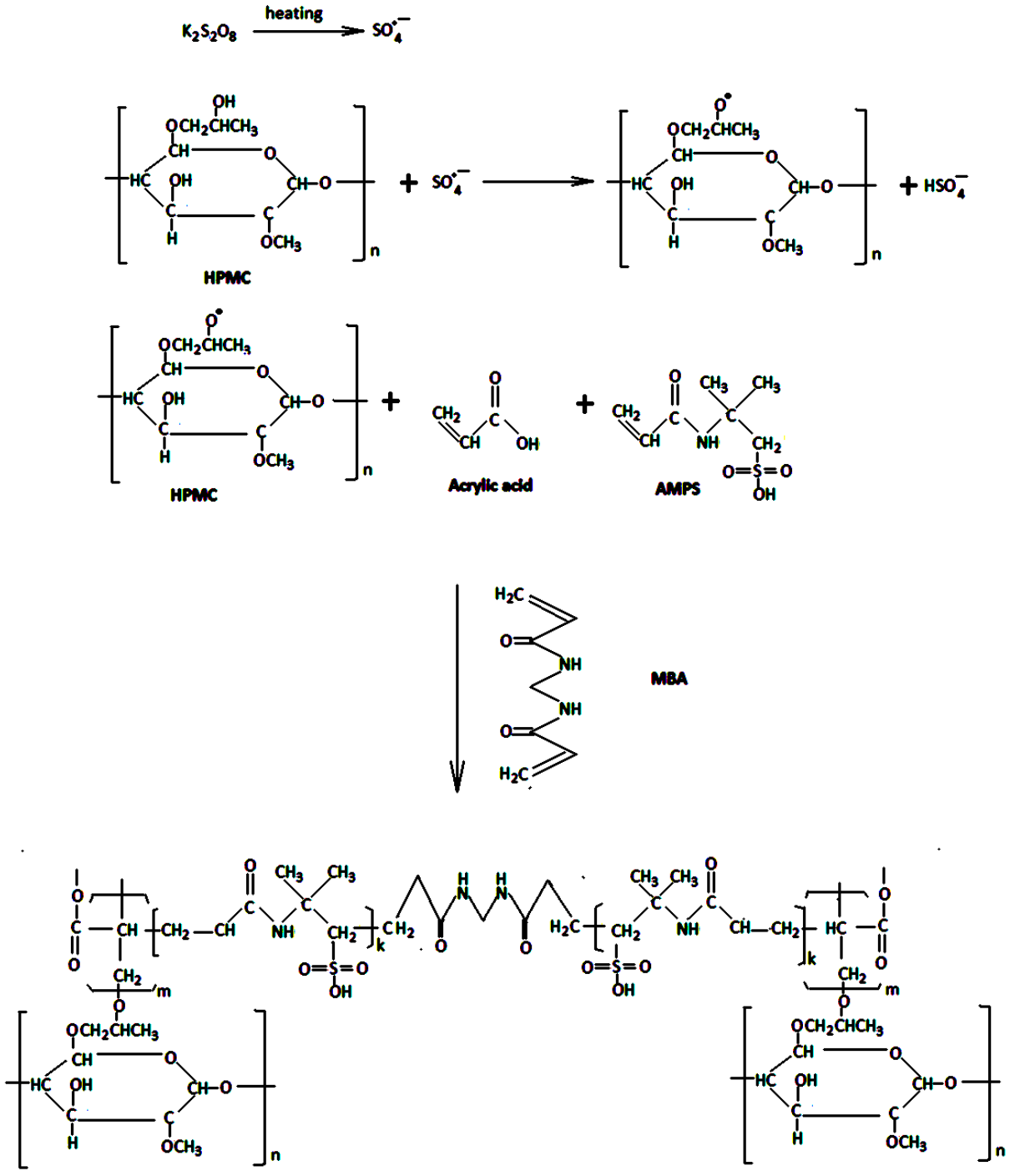

Figure 1. Crosslinked HPMC-g-poly (AA-co-AMPS) Hydrogel

4.3 *In vitro* Evaluation

4.3.1 Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectrophotometer (Bruker, Tensor 27) was used to record the spectra of hydrogel, HPMC, acrylic acid and AMPS. The hydrogel samples were ground by the help of cutter as well as pestle and mortar. The components and crushed hydrogel samples were then analysed in wavelength range of 4000 to 500 cm^{-1}.

4.3.2 Scanning Electron Microscopy (SEM)

SEM images were taken to investigate the surface morphology of super-absorbent hydrogels using a scanning electron microscope (Quanta 250, FEI). Both drug free formulations and drug loaded samples were ground and scanned at different magnifications to observe the microscopic surface of dried hydrogels. It is therefore to assess the capability to adsorb and entrap the drug into their polymeric network.

4.3.3 X-Ray Diffraction (XRD)

X-Ray Diffraction analysis determines the crystalline and amorphous properties of the substances. It investigates the interaction of components or polymers and drug. Xpert Pro diffractometer (Panalytical) diffractometer used to record x-ray diffraction. The XRD patterns of pure drug and drug loaded formulation were measured at room temperature by scanning at angle 5-50° (2 Theta), scanning speed of 20/ min^{-1}.

4.3.4 Thermal analysis

Thermal analysis was recorded by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) using Q5000 series (TA instruments) and Q2000 series (TA instruments), respectively. The hydrogel samples were crushed into powder form using pestle and mortar and passed through a mesh no. 50.

TGA

For measuring TGA, 1- 4 mg of ground sample was placed in platinum pan connected to microbalance and heated till 500°C at a rate of 20°C/min in nitrogen atmosphere.

DSC

To record DSC, hydrogel samples (1 to 3mg) along with HPMC, AMPS and acrylic acid were placed in aluminum pan crimped with an aluminum lid and heated from 0-500°C at the same rate used for TGA.

4.3.5 Swelling Study

The swelling of hydrogels was measured at different pH (2, 4.5 and 7.4) at room temperature. Dried discs of hydrogels were accurately weighed and immersed in swelling medium i.e. 0.1 M USP phosphate buffer solution. Hydrogel discs were weighed at regular intervals of time and before weighing they were placed on filter paper to remove excess of solution from the surface. The hydrogels were weighed for a period until they attain equilibrium. The swelling ratio was calculated as:

$$S = \frac{W_s}{W_d} \qquad (1)$$

Where, w_s is the weight achieved after swelling and w_d denotes the weight of dry hydrogel discs. The percentage equilibrium swelling was determined by equation given below:

$$\%ES = \frac{W_{eq} - W_d}{W_{eq}} \qquad (2)$$

Where, w_{eq} is the equilibrium weight and w_d is the initial weight of hydrogels before swelling study.

4.3.6 Drug loading

Hydrogels were loaded with drug (captopril) using absorption method by immersing the dry discs of hydrogels in 100mL captopril solution (1% w/v) comprising of phosphate buffer solution and methanol (50:50). The hydrogels were swollen till they achieved equilibrium, then taken out and dried in oven at 40°C to their constant weights. The amount of drug loaded in hydrogels was measured by extracting them with the methanol/ phosphate buffer solution in the same ratio used for drug loading. The extraction was done repeatedly at regular intervals and each time with freshly prepared solution until no drug remains in the

extracting solution. All samples of drug solutions used during extraction procedure were analyzed for drug contents. The calibration curve of captopril was drawn by preparing its various dilutions to determine the drug concentration spectrometrically at λ_{max} of 205nm.

4.3.7 Drug release

Drug release measurement was carried out by dissolution process using 0.1 M USP phosphate buffer solutions of lower and higher pH values (pH 2 and pH 7.4). The dried hydrogel discs loaded with captopril were placed in 500 ml buffer solution (dissolution medium) maintained at 37°C, agitated by a paddle stirrer at a speed of 50 rpm. Then, the samples were taken at specific time intervals and drug released was measured by UV-spectrophotometer at λ_{max} of 205nm.

4.3.8 Drug release kinetics

Various drug release models were used to determine the mechanism of drug release as given below:

Zero order kinetic models

It relates the drug delivery systems, where the rate of drug release does not exhibit concentration dependency. It is represented as:

$$M_0 - M_t = Kt \qquad (3)$$

Where, M_0isthe initial quantity of drug, M_t is the fraction of drug released at time t and K is proportionality constant.

First order kinetic models

The first order kinetics describes the concentration dependent release of drug and is represented by the following equation:

$$Log\ M_0 - Log\ M_t = K_1 t/2.303 \qquad (4)$$

Where, M_0 is the initial amount of drug, M_t is the drug concentration released at time t and K_1release constant.

Higuchi Model

Higuchi model can be presented by a simplified equation as:

$$Q = K_H t^{1/2} \qquad (5)$$

Where, Q represents the fraction of drug released at time *t* and K_H is Higuchi constant.

Weibull model

The dissolution and release process was described by an equation expressing the fraction of drug accumulated 'M' in dissolution medium at time *t* given as:

$$M = 1 - \exp\left[\frac{-(t - T_i)^b}{a}\right] \qquad (6)$$

Where,*a*defines the dependency on time, *b* denotes the shape parameter of dissolution curve and the other parameter 'T_i'represents the lag time before dissolution process.

Korsmeyer- Peppas model

Korsemeyer-Peppas model is described by a simple empirical equation to describe the Fickian and non-Fickian drug release from polymeric drug carriers, given as following:

$$M_t/M_\infty = K\, t^n \qquad (7)$$

Where, 'K' is kinetic constant that incorporates the geometric and structural properties of the hydrogels and other drug carriers. M_t/ M_∞ represents the drug fraction released at time*t* and *n*is release exponent. When the value of n is 0.45, it indicates Fickian release order and for n = 1, represents case II transport mechanism. On the other hand, n value between 0.45 and 1 corresponds to non- Fickian diffusion.

4.4 Results and Discussions

4.4.1 FT-IR Spectroscopy

The structure and formation of cross-linkageamong the polymers were investigated by spectra recorded using FT-IR spectroscopy as presented in figure 2. The spectrum(b) of HPMC shows an absorption band at 3444.60 cm^{-1} is assigned to stretching frequency of the hydroxyl (-OH) group. Another band at 1373.63 cm^{-1} is due to bending vibration of –OH. Other stretching vibration bands related to C-H and C-O were observed at 2929 cm^{-1} and 1055.52 cm^{-1}, respectively.

Spectrum (c) and spectrum (d) represents the FTIR pattern of acrylic acid and AMPS, respectively. Acrylic acid shows a characteristic peak nearly 1700cm^{-1} due to presence of carboxylic group. The spectrum of AMPS showed a band at 1666.03 cm^{-1} related to stretching of amide link (–CONH), another band at 1550.03 cm^{-1} corresponds to bending vibration of N-H and 1126.54 cm^{-1} was due to stretching vibration of -SO_3H groups. Another peak at 623.02 cm^{-1} in AMPS spectrum was also related to the -SO_3H group.

In figure 2,the spectrum (a) of HPMC-g-poly(AA-co-AMPS) suggests the formation of intermolecular hydrogen bonding due to carboxylic acid groups of acrylic acid as observed by the appearance of an absorption peak at 1710.75 cm^{-1}that was not present in individual spectra of AMPS and HPMC. The hydrogel spectrum (a) indicates the shifting of –OH vibration band of HPMC from 3444.60 cm^{-1} to 3292.58 cm^{-1} due to formation of hydrogen bonds. Hence, it confirms the crosslinking of HPMC with acrylic acid involving the reaction of –OH (of HPMC) with –COOH (of acrylic acid). In addition, characteristic bands at 1547.30 cm^{-1} (C=O stretching vibration of –CONH groups) and 1153.14 cm^{-1} were attributed to stretching vibration of sulfonate (–SO_3H) groups of AMPS monomer. The results indicated successful grafting of monomers (acrylic acid and AMPS) onto HPMC polymer chains. These peaks observed were according to the FTIR spectrum noticed by Wang *et al.*[313] however slight variation was due to interaction of acrylic acid and AMPS with functional groups of polymers.

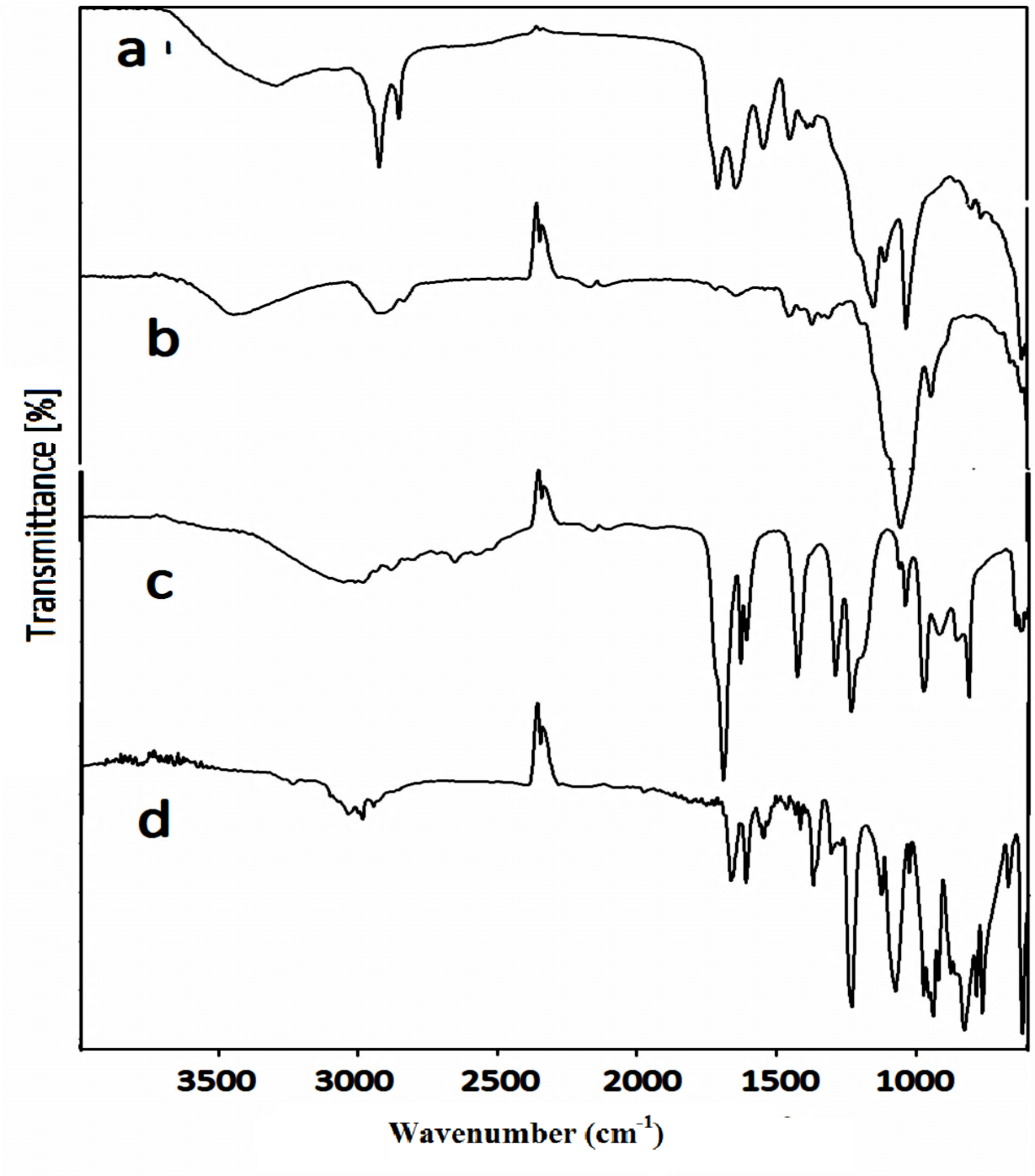

Figure 2. FT-IR Spectra of HPMC-g-poly (AA-co-AMPS) (a), HPMC (b), Acrylic acid (c) and AMPS (d)

4.4.2 SEM

In order to determine the microstructure and surface morphology of hydrogel formulations, SEM images were taken. Scanning electron microscopy is one of the preferred methods to characterize the hydrogels in terms of porosity and water retention. The micrographs recorded as shown in figure 3 (A and B) show the surface of the drug free and drug loaded hydrogels.

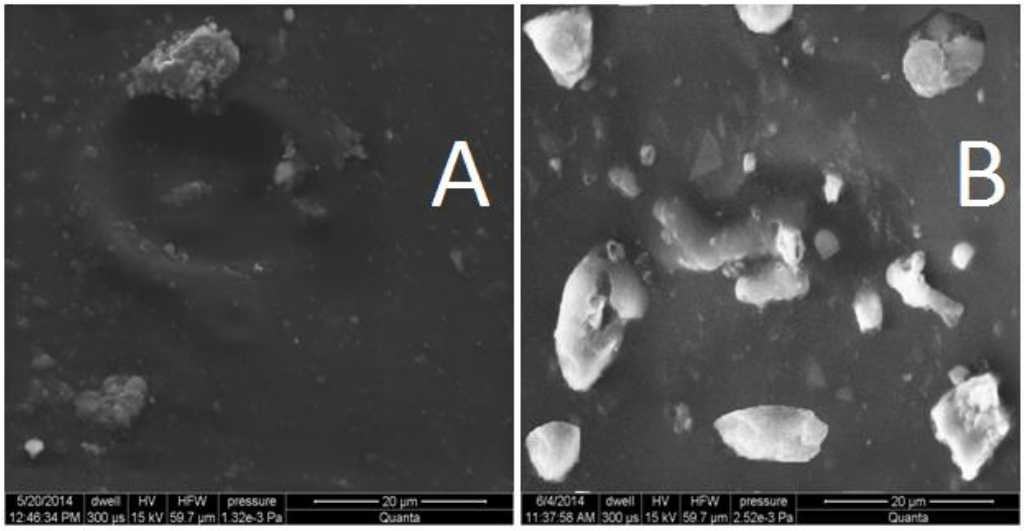

Figure 3. SEM images of HPMC-g-poly (AA-co-AMPS) hydrogels, without drug (A) and loaded with drug (B).

The SEM images in figure 3 clearly show that hydrogel surface was rough along withmicro-spaces for water retention and entrapment ofsolutes. Figure 3 (A) presents drug free hydrogel formulation with voids and spaces for accommodation of biological fluids as well as drug particles. Theyhad tendencyto exhibit remarkable swelling because of their water absorption capability. Moreover, due to the presence of these voids and roughness of surface of hydrogels, the captoprilwasloaded into these regions as shown in figure 3 (B).It was indicated from the prepared grafted polymeric network HPMC-g-poly (AA-co-AMPS) had ability to act as suitable drug carriers for drug delivery.

4.4.3 XRD

X-ray diffractograms of pure captopril and captopril loaded hydrogel formulation are presented in figure 4. The diffraction patterns of hydrogels loaded with drug were compared with pure drug. The XRD scan of plain captopril had characteristic sharp and intense peaks between 0° and 50° (2θ), which were appeared due to its crystalline nature as shown in figure 4, diffractionpattern (c).

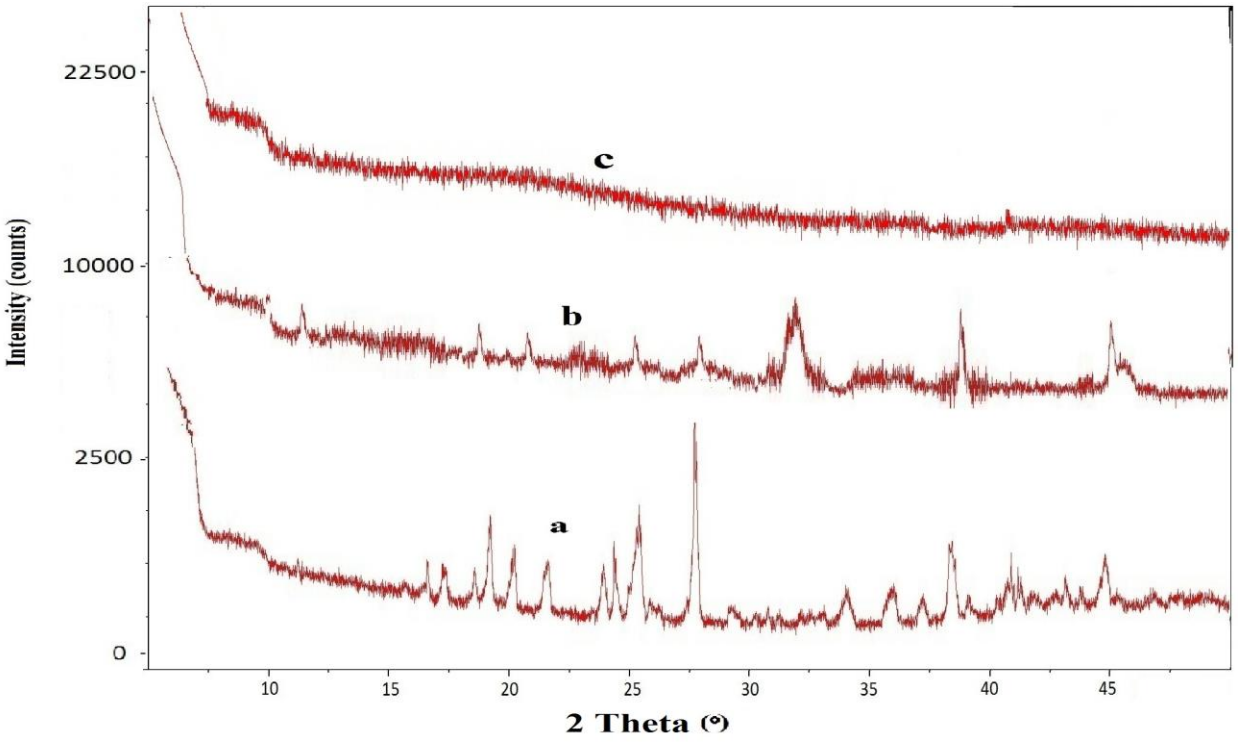

Figure 4. XRD patterns of captopril (a) and Drug loaded HPMC-g-poly (AA-co-AMPS) hydrogel (b) Drug free HPMC-g-poly (AA-co-AMPS) hydrogel

The appearance of a sharp peak at 2 θ= 27.79° is characteristic of captopril. It could be seen in diffraction pattern a, that no peak was observed in cross-linked copolymer. However, diffractogram of drug loaded hydrogel was dense like that of drug free hydrogelbutshowing peaks with low intensity. Therefore, in comparison to pure captopril, the captopril loaded formulations had low intensity and dense peaks suggesting the amorphous distribution of drug into polymeric network, as it could be observed from figure 4 diffractogram (b). This observation iscorresponding with XRD analysis studied by Rao *et al.*[314], where drug was molecularly dispersed in the polymeric matrix of poly(acryl amide-co-2-acrylamido-2-

methyl-1-propanesulfonic acid-co-acrylamidoglycolic acid) hydrogels. Similar results had also beennoted by Kim *et al.*[315]

4.4.4 Thermal analysis

The grafting of HPMC-g-poly (AA-co-AMPS) polymeric network was also determined by theromogravimetric analysis (TGA). The pure acrylic acid decomposition starts at nearly 80°C and complete mass loss was observed at 189.50°C as shown in figure5, thermogram (A). Similarly, the degradation of other monomer AMPS had taken lesser time as can be seen in thermogram (B), figure 5. The TGA thermogram (C) of HPMC shows decomposition at 265°C which then continued till 350°C, during that period 78% of loss in weight was observedbecause of polymer degradation.

In comparison to the individual components, thermogram of the grafted product (D) was recorded, where three stages of decomposition from 30°C -500°C were observed. In the first stage of degradation, loss of weight started from 114°C to 25°C, there was about 12% weight loss during this stage due to loss of absorbed and bound water. Then second stage of weight loss started at 250°C and continued to 331.88°C, corresponding to 40% weight loss. Finally the third stage beginning from 332°C till 500°C caused the degradation of hydrogel's structure. In case of HPMC, there was complete removal at 490°C, whereas the hydrogel had 67% weight loss and 33% still remaining at that temperature. A greater thermal stability of the formed polymeric network was observed as compared to its individual polymer and monomers (HPMC, acrylic acid and AMPS). Hence, the hydrogels prepared were more stable and resistant to higher temperatures.

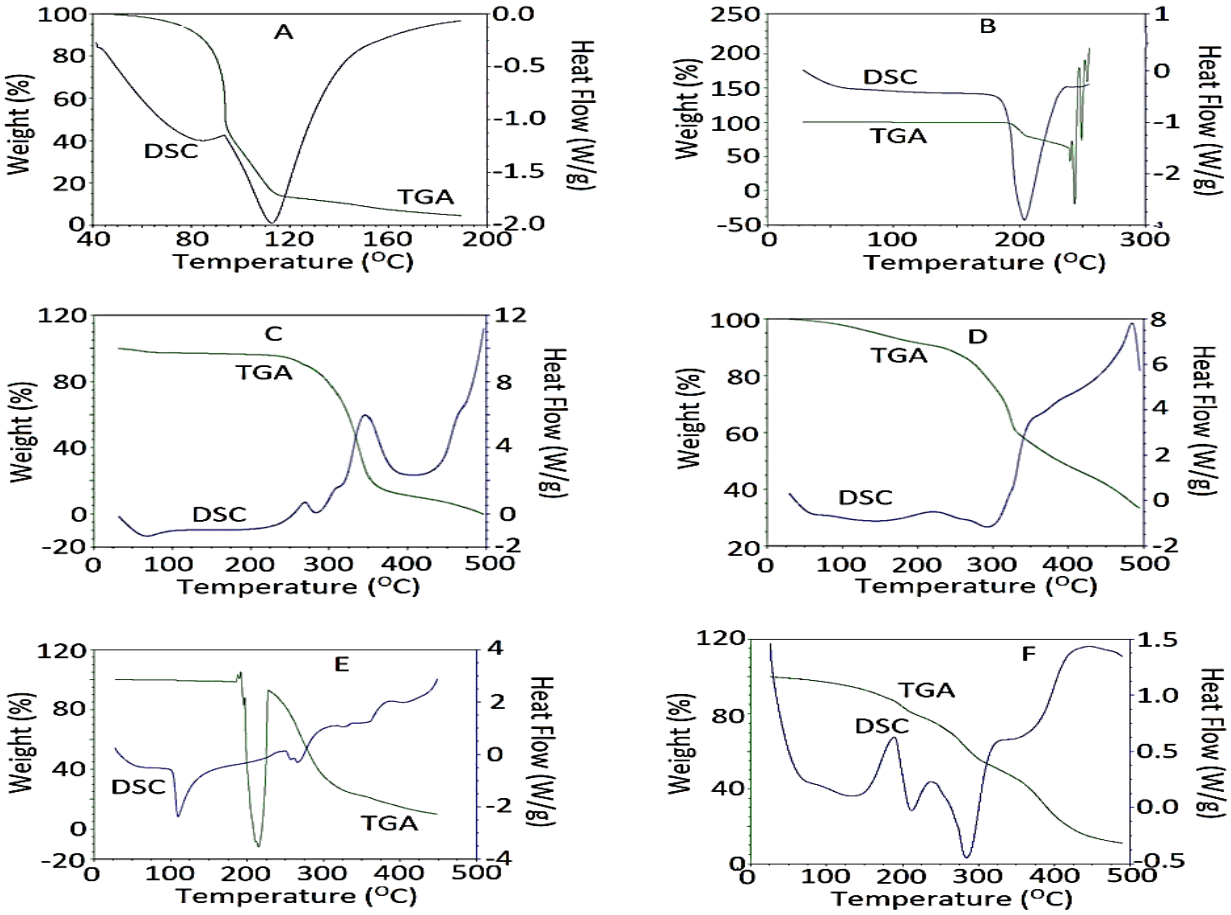

Figure 5. DSC and TGA thermograms of acrylic acid AA (A), AMPS (B), HPMC (C), drug free hydrogel (D), captopril (E) and drug loaded hydrogel (F)

The alterations in heat capacity as well as enthalpy changes are measured using Differential Scanning Calorimetry (DSC). It is a well-known and established technique adopted for quantitative assessment of physicochemical variations in heat capacity of crystalline drugs, when loaded into hydrogels. The DSC endothermic peaks of pure HPMC, AA, AMPS and cross-linked polymeric network were in accordance with TGA thermal patterns. The thermal behavior of the pure captopril, drug loaded S3 hydrogel was characterized using DSC, as shown in Figure 5 (F).The disappearance of characteristic peaks and appearance of other peaks suggests some sort of interaction of drug with polymers. Thermogram (E) presenting DSC pattern of pure captopril, shows the appearance of sharp peak at 106°C indicating melting point of captopril. This peak of drug

was not appeared in DSC thermogram of the drug loaded formulation, whereas two new peaks were observed at 225°C and 292°C.

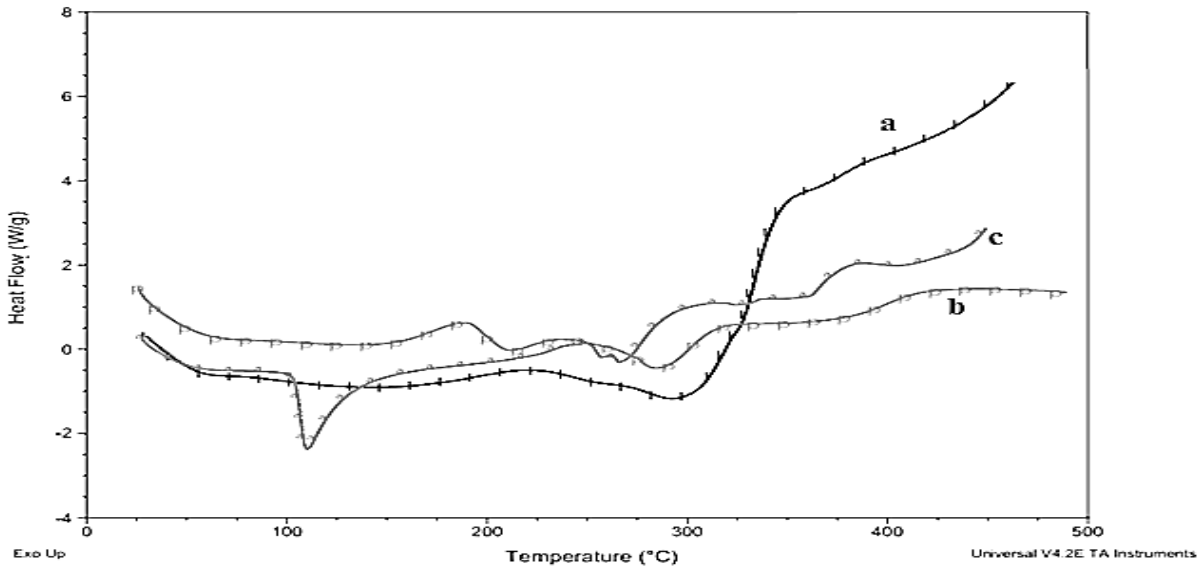

Figure 6. DSC thermograms of a) drug free and b) drug loaded and c) pure drug

It could be clearly suggested that the drug loaded Hydrogels showed an increase in the exothermic peak temperature (Figure 6). The extra obvious peak of drug (106 °C) was not observed in drug loaded hydrogels. It suggests that captopril was molecularly dispersed in different hydrogel matrix without changing its thermal behavior, indicating the stability of drug and drug loaded hydrogel formulation.The resultant DSC analysis isaccording to observations of Kim *et al.*[315]and Manjanna *et al.*[281]

4.4.5 Swelling Study

Swelling ratios of the synthesized gels were measured gravimetrically at the pH from 1.2, 4.5 and 7.4 in phosphate buffer. The swelling of hydrogels is dependent upon the presence of hydrophilic groups. Hydroxypropylmethylcellulose interact with water due to its –OH groups. The hydrogel swelling is enhanced by increasing the amount of AMPS. This high swelling property of AMPS is attributed to the presence of strongly ionizable sulfonate groups that create charge repulsion among the grafted chains. The sulfonate groups present have better hydrophilicity than carboxylate groups. The carboxylic groups associated with acrylic acid impart a pH responsiveness that prevents the abrupt swelling of copolymeric

system due to AMPS. Increasing the pH of buffer solution made ionization of carboxylic groups, which ultimately generates repulsive forces responsible for swelling behaviour of hydrogels containing acrylic acid.

Effect of polymer concentration

Hydrogels containing higher amount of polymer show lesser comparative swelling ratios As shown in figure7, S3 comprising lower amount of polymer swell more as compared to S6 and S7 formulations. As the quantity of polymer increases in the hydrogels, the value of their swelling ratio decreases.

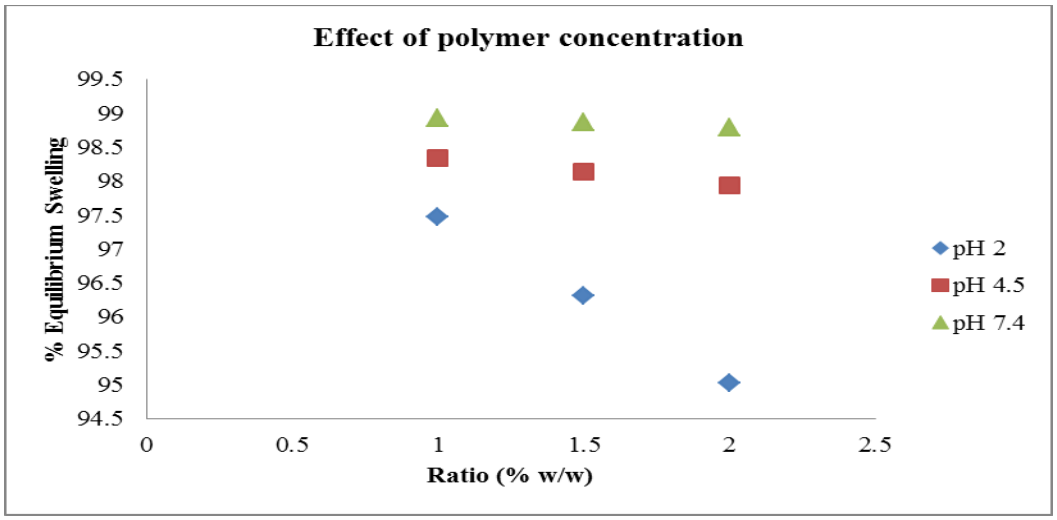

Figure 7. Percent equilibrium swelling (%ES) of formulations at different pH containing different concentrations of HPMC

Effect of Cross-linking agent

The graphic presentation of swelling ratios in figure 8 (a) and percent equilibrium swelling in figure 8(b) elaborates the effect of different concentrations of MBA on swelling behavior. By increasing the amount of crosslinking agent there was decrement in swelling ratio. Increasing the crosslinker amount leads to increment of crosslinking density due to higher interaction among the components and ultimately reduces the porosity. Hence, the structure of hydrogels become dense having lesser spaces to accommodate aqueous solutions. On the other hand, the hydrogels comprising less amounts of MBA were able to swell more due to lower crosslinking density. The formulation S10 containing the lower quantity of crosslinker

exhibited highest swelling ratio among other formulations.Various quantities of MBA, 0.09% w/w, 0.12% w/w and 0.15% w/w were added for S3, S8 and S9, respectively. S3 containing lesser amount of crosslinking agent exhibit higher percent equilibrium swelling (% ES) as shown in figure 8 (b).

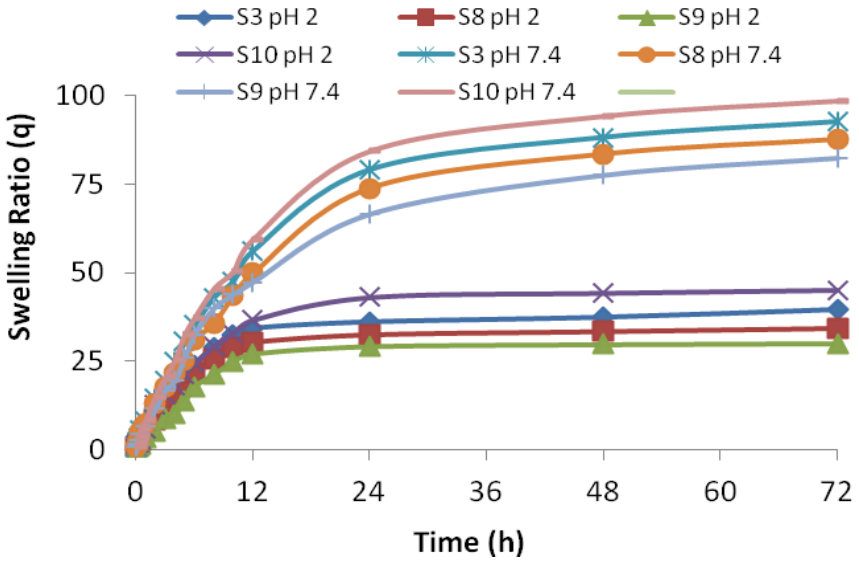

Figure 8(a). Comparative Swelling ratios of the hydrogels with different concentrations of crosslinking agent

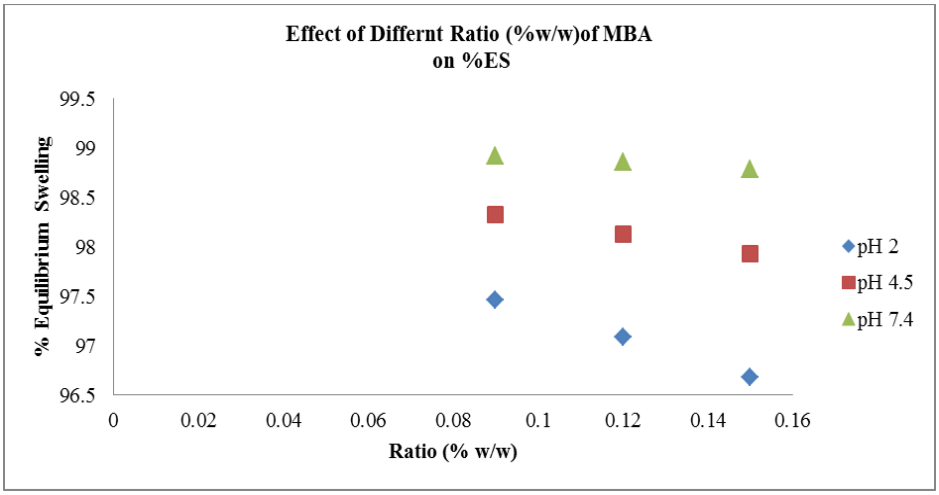

Figure 8(b). Percent equilibrium swelling (%ES) of formulations at different pH containing different concentrations of MBA

Effect of AMPS concentration

The result of varying the ratio of acrylic acid to AMPS is demonstrated in Figure 9 (i) and 9 (ii). By increasing the amount of AMPS in formulation enhanced the swelling, which reached maximum as the mass ratio of AA to AMPS was 4:1. Further increase of AMPS content led to a decrement of swelling ratio. By increasing the amount of AMPS, number of hydrophilic groups such as -CONH and -SO_3H increased accordingly. As a result, the synergistic effect produced by-CONH and -SO_3H on AMPS, together with that of -COOH on AA increased,[316] which brought about great enhancement of absorbency of aqueous solution.

However, when the mass ratio of AA to AMPS was below 4:1, which mean that AMPS content is over one-fifth of total monomers, swelling decreased in respect that the electrostatic repulsion between ions weakened and the three-dimensional network compacted. In addition, as AMPS contained quaternary carbon atoms and -SO_3H groups which were enormous in size, stretching of polymer chains would be obstructed and absorbency would decrease accordingly. The subsequent decrease in swelling ratio of hydrogels can be ascribed to low reactivity of AMPS monomer.[317,318]This indicates that the monomer grafting onto CMC chains decreases with increasing AMPS/AA molar ratio above a certain limit.

The swelling of formed polymeric network with various ratios of acrylic acid and AMPS was relevant to that observed by Zhu *et al.,*[319] where the absorption of aqueous solution was dependent upon optimum concentration of AMPS. Further increase in AMPS led to decrease in water absorption characteristics and ultimately swelling of hydrogel. However, it was varying from results obtained by Zhang *et al.*[320] in terms of swelling behavior with higher contents of AMPS than acrylic acid, which could be due to interaction of AMPS with polymer i.e. Xylan. In our case the crosslinking was mainly due to interaction of functional groups of AMPS and acrylic acid(sulfonic groupsand carboxylic groups) which were then grafted on backbones of HPMC. Hence, the swelling characteristics were dependent upon the optimum ratio of both monomers in polymeric network.

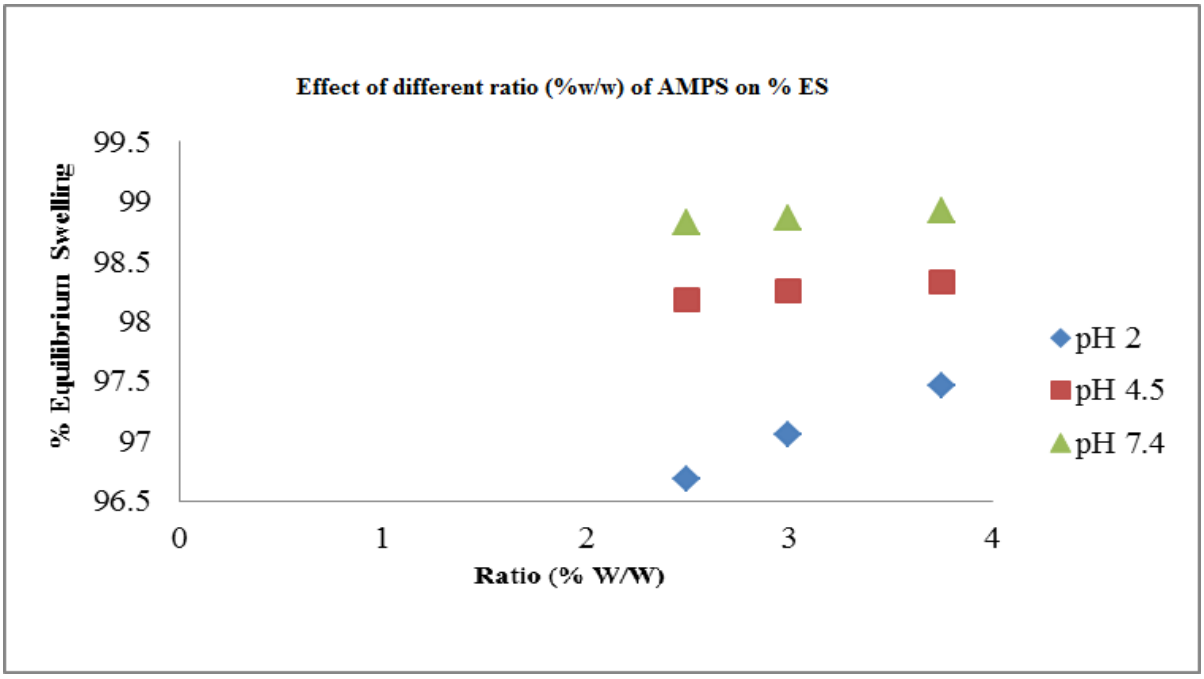

Figure 9(i). Increase in percent equilibrium swelling with increase in AMPS Concentration

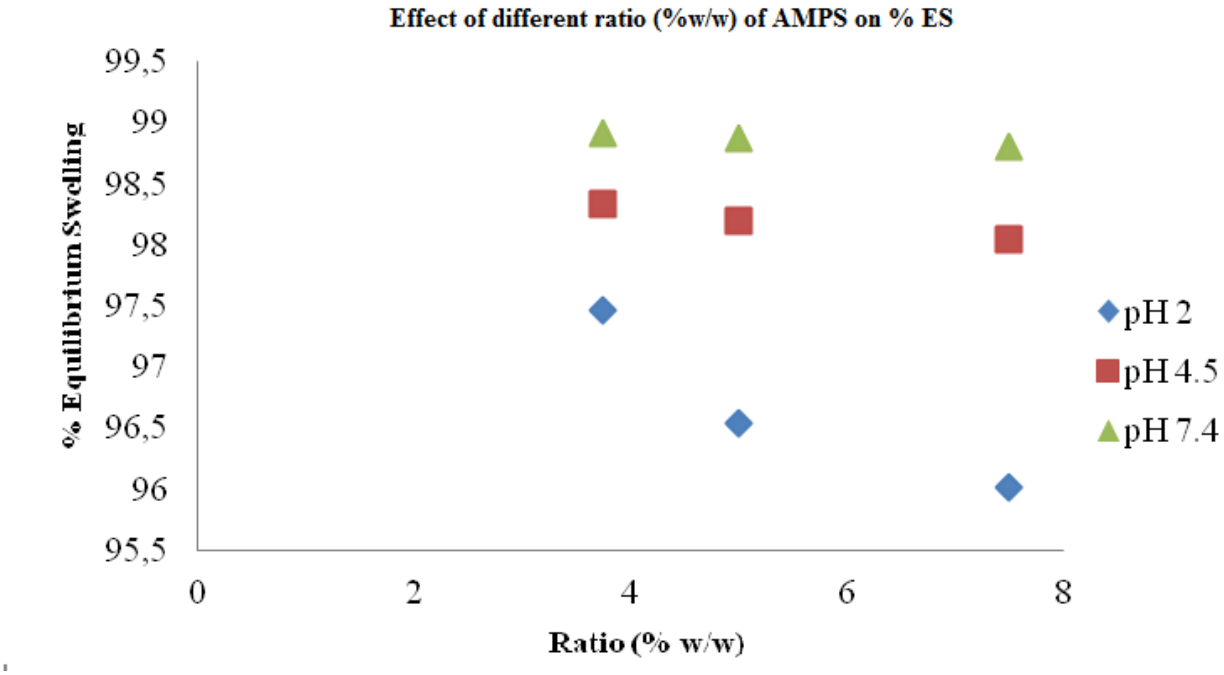

Figure 9(ii). Decrease in percent equilibrium swelling(%ES)with increase in AMPS concentration above 3.75 g (with AA/AMPS ratio 4:1)

4.4.6 Drug loading and release studies

The hydrogel discs exhibiting greater swelling accommodated higher amounts of drug. The release study of captopril was performed at pH 2 and pH 7.4 as shown in figure 10. The drug release at both pH (2 and 7.4) was observed for a period of 24 hrs, where USP phosphate buffer was used as dissolution medium. Drug release measured was in correspondence to swelling studies where relatively more amount of drug was loaded and ultimately released in formulations exhibiting more swelling.

Table 2. Amount of Captopril loaded and percentage of drug released at pH 2 and pH 7.4

Formulation code	Amount of captopril loaded (mg) per 0.3 gram of dry hydrogel discs	% release of captopril	
		pH 2	pH 7.4
S1	113.5	32.83	82.57
S2	118.81	34.75	86.28
S3	124.28	38.22	88.50
S4	117.5	33.29	84.39
S5	109.7	30.84	81.56
S6	112.55	32.63	82.12
S7	104.15	28.65	77.68
S8	119.56	35.16	87.07
S9	114.31	33.08	83.16
S10	127.78	44.40	90.01

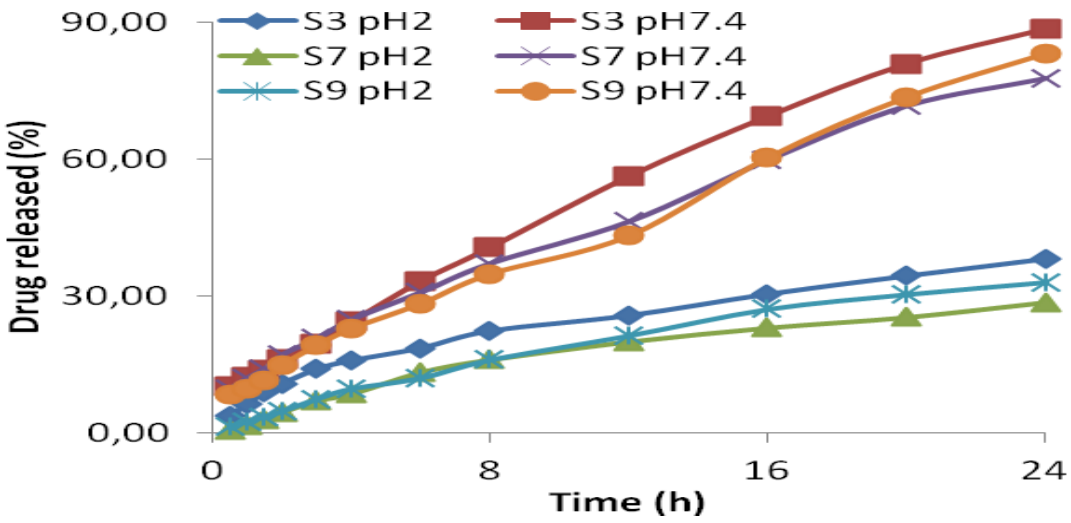

Figure 10. Captopril released up to 24 h from HPMC-g-poly (AA-co-AMPS) hydrogels (S3, S7, and S9) in dissolution media of pH 2 and pH 7.4

The drug release kinetics of prepared superabsorbent hydrogels was determined by different kinetic models already mentioned. The values of release coefficient R^2calculated by kinetic models are presented in table 3. The drug release from hydrogel formulations were best fitted into the kinetic model having value of R^2 close to 1.

The release exponent*n*obtained byKorsemeyer-Peppas modelfor the formulations of HPMC-g-poly (AA-co-AMPS) copolymer containing different components, polymer concentration, AA/AMPS ratio and amount of crosslinker are presented in table 4.

Table 3. Determination coefficient (R^2) of various drug release kinetic models for the prepared superabsorbent hydrogel formulations

Sample code	pH	Zero order	First order	Higuchi	Weibull
S1	2	0.9815	0.7443	0.9954	0.7489
	7.4	0.9934	0.6024	0.9673	0.8986
S2	2	0.9629	0.6954	0.9959	0.7257
	7.4	0.9956	0.5858	0.9659	0.9198
S3	2	0.9484	0.5329	0.9961	0.8081
	7.4	0.991	0.5774	0.9822	0.8989
S4	2	0.9691	0.7125	0.9974	0.7001
	7.4	0.9945	0.5842	0.9683	0.9133
S5	2	0.9717	0.7269	0.9978	0.7025
	7.4	0.9855	0.5106	0.9931	0.8856
S6	2	0.9851	0.7503	0.9919	0.7142
	7.4	0.9912	0.5766	0.9816	0.8894
S7	2	0.9434	0.6957	0.9947	0.6683
	7.4	0.9917	0.5428	0.9842	0.901
S8	2	0.9633	0.6986	0.9941	0.7366
	7.4	0.9958	0.5912	0.9637	0.9188
S9	2	0.9758	0.7307	0.9938	0.7743
	7.4	0.9956	0.6003	0.9691	0.987
S10	2	0.9359	0.4794	0.9845	0.7975
	7.4	0.994	0.5914	0.9775	0.9071

Table 4. Mechanism of drug release by determination of release exponent 'n'

Sample code	pH	N	R
S1	2	0.59	0.9954
	7.4	0.72	0.9855
S2	2	0.47	0.9914
	7.4	0.73	0.9871
S3	2	0.56	0.9962
	7.4	0.59	0.9989
S4	2	0.55	0.9957
	7.4	0.69	0.987
S5	2	0.63	0.9957
	7.4	0.58	0.9979
S6	2	0.79	0.9977
	7.4	0.64	0.9869
S7	2	0.54	0.9795
	7.4	0.63	09952
S8	2	0.46	0.991
	7.4	0.78	0.9853
S9	2	0.48	0.9956
	7.4	0.78	0.987
S10	2	0.61	0.9757
	7.4	0.62	0.9991

The mechanism of drug release was indicated by the values of n i.e. release exponent. By fitting of recorded data to Peppas model, it was investigated that approximately all hydrogel formulations in spite of using different concentrations of polymer, monomers and crosslinking agent were following non- Fickian mechanism of drug release as presented in Table 4. The value of n in all cases were more than 0.45 but lesser than 0.85.

CONCLUSIONS

The results and discussion reveal that the developed superabsorbent hydrogel exhibit remarkable swelling properties, reasonable stability and smart pH responsiveness. The HPMC-g–poly (AA-co-AMPS) polymeric network was successfully crosslinked by a well-established and widely used chemical crosslinking method, free radical polymerization. The formulations prepared by varying amounts of components were then loaded with an antihypertensive drug, captopril. The drug entrapped into these hydrogels remained stable and was compatible with its components. The hydrogel network was also capable to release relatively smaller fraction of drug in acidic medium and more quantity at higher pH. Thus, after oral administration, the hydrogel formulation would be capable of exerting the effects throughout its retention in the stomach and intestine. Therefore, this can prove its worth as a successful and promising drug carrier for the controlled release of captopril, which can be used for the treatment of hypertensive patients as well as for the management of cardiac disorders.

Chapter no.5

Poly(vinyl alcohol)-co-poly(2-acrylamido-2-methyl-1-propane sulfonic acid) gastro-retentive hydrogel by microwave radiation

Summary

Background of the Study

Hydrogel possessing highly swollen characteristics was prepared by crosslinking of poly(vinyl alcohol) (PVA) with 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) as a drug carrier of Captopril.

Methods

Polyvinyl alcohal was crosslinked with AMPS by microwave radiation using N,N'-methylenebisacrylamide (MBA) as crosslinker and very low quantities of potassium persulfate (KPS) as initiator. The captopril was loaded as model drug and its concentration was measured at wavelength of 205 nm using UV spectrophotometer. The swelling studies were performed at pH 2 and pH 7.4 to determine variation in water retention at low and higher pH. This super-absorbent polymeric system was evaluated by FT-IR, SEM, XRD, and thermal analysis (DSC and TGA).

Results

The crosslinkage of components and synthesis of hydrogel were confirmed by FT-IR, XRD, TGA and DSC analysis. The hydrogel formulations with higher contents of AMPS and appropriate dose of radiation had shown maximum swelling. Drug release observed was relatively higher at pH 2 than at pH 7.4, because of more swelling capacity of AMPS at lower pH of the aqueous medium.

Conclusions

It was concluded from the current research work that a polymeric device containing polyvinyl alcohol and AMPS was developed successfully under the effect of microwave radiations. The prepared superporous hydrogel could be a potential candidate as drug carrier.

Keywords: Composite, Superabsorbent, Polymerization, 2-Acrylamido-2-methyl-1-propane sulfonic acid, polyvinyl alcohol, Initiator, Cross linker, Hydrogel.

5.1 Introduction

In current era of science and technology, the swellable polymeric gels are the subject of keen interest among the research groups due to diversity of their applications in various areas of pharmaceutical and biomedical fields.[321] Hydrogels based on natural polymers are often preffered due to their better bioinertness, but they have a drawback of less mechanical strength and higher costs.[322-328] In comparison, using synthetic polymers overcomes these shortcomings and imparts a reasonable strength to polymeric networks. Among them, PVA is most commonly used polymer for preparing various hydrogels such as superabsorbants, semi-interpreting networks and superporous hydrogels.[329-331] It is a synthetic hydrophilic polymer, which exhibit reasonable swelling capability, while maintaining stability of the formulation. Other advantages are its non-toxicity, biocompatibility, physical and chemical stability. Due to these properties they have been extensively used in controlled release drug delivery as well as in medical devices such as implants, artificial pancreas, nanofilteration and hemodialysis.[332-336]

Many hydrogels prepared containing polyvinyl alcohol in combination with acrylic acid or methacrylic acid do not exhibit rapid swelling properties, thus, providing slow drug release.[337-339] In contrast, some situations desire a fast release of drug, by attaining higher swelling in shorter time, for example, Super porous hydrogels. A gastro-retentive drug delivery hydrogel was prepared by PVA and chitosan for controlled release of rosiglitazone maleate.[340] In the present work, a hydrogel with rapid and high swelling characteristics was prepared to entrap an antihypertensive drug; Captopril, for the treatment of hypertension. To develop gastroretentive system, PVA was crosslinked with 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), a hydrophilic monomer with strongly ionizable sulfonate groups, having tendency to swell at all pH ranges.[341-344] It imparts high swellability to hydrogels at low pH that could be retained in stomach for longer periods of time, thus releasing the drug in acidic medium. This will have a benefit regarding the stability of captopril which remains more stable at acidic pH.

To fulfill the objectives of developing a porous hydrogel network, the PVA was crosslinked with AMPS via microwave radiation method. Under the influence of microwaves, highly porous structures are formed with uniform crosslinking among the polymeric chains.[72]

Microwaves have ability to initiate crosslinking among polymers, hence very low quantity of initiator is required.[267,274] The hydrogels were synthesized by exposure to different doses of radiation and varying initiator concentration. They were characterized by FTIR, SEM, and XRD, thermal analysis (DSC and TGA) as well as swelling and drug release were also measured.

5.2 MATERIALS & METHODS

5.2.1 Chemicals

PVA, MW 72000 (Applicap), AMPS, (99%, Aldrich-product of Germany) N,N-Methylene-bis-acrylamide (98%, Fluka-Switzerland), potassium persulphate (AnalaR, BDH-England), and potassium dihydrogen phosphate (Merk- Germany) were purchased through local commercial sources. Distilled water was used while produced in the labortary and solvents of analytical grades were used.

5.2.2 Hydrogel Synthesis

Poly(vinyl alcohol) 1-3% W/W was dissolved in distilled water by continuous stirring at 80°C for 1 hour. The hot solution was brought to reduced temperature (60°C) and different low concentrations (0.05- 0.25% W/W) of initiator were used and stirred for 15 minutes. Then, the temperature was further decreased and cooled down to 30°C and finally crosslinker (MBA 1 mol % of monomer concentration) was added along with monomer (AMPS) solution. The whole mixture was transferred in a glass tube and placed on turntable of Electrolux domestic microwave oven. Various batches of hydrogels were prepared by exposing the material at different doses of microwave radiations at electric power of 100 W, 180 W and 300W. Then, the glass tubes were cooled to 25°C and hydrogels were taken out and cut in the form of discs of nearly 8mm long. They were then thoroughly treated with ethanol and distilled water mixture (50:50) for removing catalysts and uncross-linked monomer till the pH of solutions (which was initially ranging from pH 3-3.5) after washing becomes nearly same as pH of washing solution before being used i.e. pH 6.5 to 7. After washing process, the hydrogel discs were air dried for overnight and then transferred to oven at 45°C for 4 to 5 days until they attain a constant weight. Figure 1 shows the crosslinking reaction of PVA and AMPS initiated by potassium persulfate (KPS) with assistance of

microwave radiation in the presence of crosslinker (MBA). Table 1 presents hydrogel formulations prepared under exposure to different radiation dose and using.concentrations of initiator, polymer (PVA), monomer (AMPS).

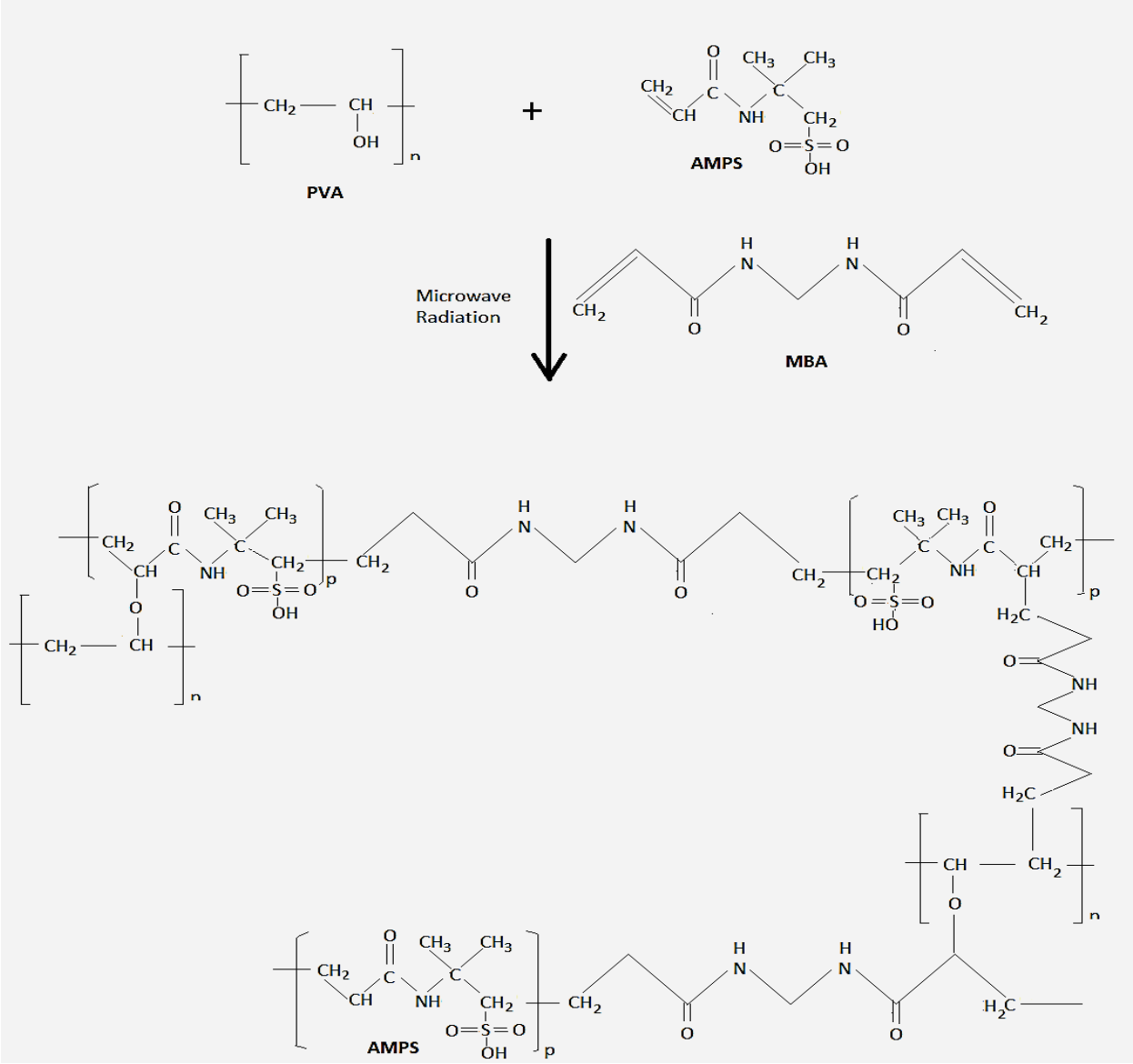

Figure 1. Crosslinking of PVA and AMPS using MBA as crosslinker under influence of Microwave radiation

Table 1. Hydrogels formulations prepared by exposure to different radiation doses, initiator concentration, amount of polymer and monomer

Sample code	Electric power (Watts)	Concentration of initiator (% W/W)	Monomer concentration (g/100g)	Polymer Concentration (% W/W)
SP1	100	0.25	25	1
SP2	180	0.25	25	1
SP3	300	0.25	25	1
SP4	300	0.15	25	1
SP5	180	0.15	25	1
SP6	180	0.05	25	1
SP7	180	0.15	20	1
SP8	180	0.15	30	1
SP9	180	0.15	25	2
SP10	180	0.15	25	3

5.3 *In vitro* Evaluation

5.3.1 Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectrophotometer (Bruker, Tensor 27) was used to record the spectra of hydrogel, HPMC, acrylic acid and AMPS. The hydrogel samples were grounded by the help of cutter as well as pestle and mortar. The components and crushed hydrogel samples were then analysed in wavelength range of 4000 to 500 cm^{-1}.

5.3.2 Scanning Electron Microscopy (SEM)

SEM images were taken to investigate the surface morphology of super-absorbent hydrogels using a scanning electron microscope (Quanta 250, FEI). Both drug free formulations and drug loaded samples were ground and scanned at different magnifications to observe the microscopic surface of dried hydrogels.

5.3.3 X-Ray Diffraction (XRD)

X-Ray Diffraction analysis determines the crystallinaty and amorphous properties of substances. It investigates the interaction of components or polymers and drug. Xpert Pro diffractometer (Panalytical) diffractometer used to record x-ray diffraction. The XRD patterns of pure drug and drug loaded formulation were measured at room temperature by scanning at angle 5-50° (2 Theta) and scanning speed of 20/ min-^{1}.

5.3.4 Thermal analysis

Thermal analysis was recorded by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) using Q5000 series (TA instruments) and Q2000 series (TA instruments), respectively. The hydrogel samples were crushed into powder form using pestle and mortar and passed through a mesh no. 50.

TGA: For measuring TGA, 1- 4 mg of ground sample was placed in platinum pan connected to microbalance and heated upto 500°C at a rate of 20°C/min in nitrogen atmosphere.

DSC: To record DSC, hydrogel samples (1 to 3mg) along with HPMC, AMPS and acrylic acid were placed in aluminum pan crimped with an aluminum lid and heated from 0-500°C at the same rate used for TGA.

5.3.5 Swelling Study

The swelling of hydrogels was measured at different pH (2, 4.5 and 7.4) at room temperature. Dried discs of hydrogels were accurately weighed and immersed in swelling medium i.e. 0.1 M USP phosphate buffer solution. Hydrogel discs were weighed at regular intervals of time and before weighing they were placed on filter paper to remove excess of solution from the surface. The hydrogels were weighed for a period until they attain equilibrium. The swelling ratio was calculated as:

$$S = \frac{w_s}{w_d} \qquad (1)$$

Where, w_s is the weight achieved after swelling and w_d denotes the weight of dry hydrogel discs. The percentage equilibrium swelling was determined by equation given below:

$$\% \text{ ES} = \frac{w_{eq} - w_d}{w_{eq}} \qquad (2)$$

Where, w_{eq} is the equilibrium weight and w_d is the initial weight of hydrogels before swelling study.

5.3.6 Drug loading

Hydrogels were loaded with drug (captopril) using absorption method by immersing the dry discs of hydrogels in 100ml captopril solution (1% w/v) comprising of distilled water and methanol (50:50). The dics were swollen till they achieved equilibrium, then taken out and dried in oven at 40°C to their constant weights. The amount of drug loaded in hydrogels was measured by extracting them with the methanol/ distilled water in the same ratio used for drug loading. The extraction was done repeatedly at regular intervals and each time with freshly prepared solution until no drug remains in the extracting solution. All samples of drug solutions used during extraction procedure were analyzed for drug contents. The calibration curve of captopril was drawn by preparing its various dilutions to determine the drug concentration spectrometrically at λ_{max} of 205nm.

5.3.7 Drug Release

Drug release measurement was carried out by dissolution process using 0.1 M USP phosphate buffer solutions of lower and higher pH values (pH 2 and pH 7.4). The dried hydrogel discs loaded with captopril were placed in 500 ml buffer solution (dissolution medium) maintained at 37°C, agitated by a paddle stirrer at a speed of 50 rpm. Then, the samples were taken at specific time intervals and drug released was measured by UV-spectrophotometer at λ_{max} of 205nm.

5.4 Results and Discussion

5.4.1 FT-IR Spectroscopy

FT-IR spectra of PVA, AMPS and grafted polymeric network were recorded as shown in figure 2. In PVA spectrum a characteristic broad band at 3286.82 cm^{-1} illustrate the O–H stretching vibration of hydroxyl group. A relatively smaller peak at 2941.43 cm^{-1} is attributed to C-H stretching of methylene group and 1089.38 cm^{-1} for characteristic – C– O stretching. The observed peaks for PVA were in accordance with that noted by Basak *et al.*[345] where 3290 cm^{-1} indicated O-H stretching vibration, 2864 cm^{-1} for C-H stretching and 1069cm^{-1} for -C-O stretching vibrations. In our work, a reduction in intensity of these stretching vibrations was observed after crosslinking with AMPS in the presence of crosslinker (MBA) and initiator. Due to reaction initiated by initiator, the double bonds in AMPS as well as crosslinker was activated, hence C=C was not remained intact and contributed in bonding during crosslinking reaction. In figure 2, the spectrum of AMPS showed a band at 1666.03 cm^{-1} related to stretching of amide link (–CONH), another band at 1550.03 cm^{-1} corresponds to bending vibration of N-H and 1126.54 cm^{-1} is due to stretching vibration of -SO_3H groups. Another peak at 623.02 cm^{-1} in AMPS spectrum is also related to the -SO_3H group.

The spectral analysis of PVA-co AMPS hydrogels shows modification in spectra of individual components due to their crosslinking resulting in the formation of new bonds. The characteristic vibrational peaks of PVA were shifted from 3286.82 cm^{-1}, 2941.43 and 1089.38 cm^{-1} to 3214.94 cm^{-1}, 2921.07 cm^{-1} and 1039.10 cm^{-1}, respectively. Another peak at 844.49 cm^{-1} was shifted to 804 cm^{-1} indicated a reduction in bending vibrations of C – H. A characteristic band in AMPS at 1666.03 cm^{-1} was reduced to 1646.27 cm^{-1} and 1550.03 was shifted to 1544.29 cm^{-1}. A peak at 1155.61cm^{-1} implies stretching vibration of -SO_3H groups of AMPS monomer. In addition, another band at 621.02 cm^{-1} was due to -SO_3H as indicated by Osorio-Fuente *et al.*,[346] that the peaks in range 620 to 625 cm^{-1} confirms the presence of sulfonic group (-SO_3H). From these FT-IR spectra, it is indicated that AMPS is polymerized and successfully crosslinked with polyvinyl alcohol.

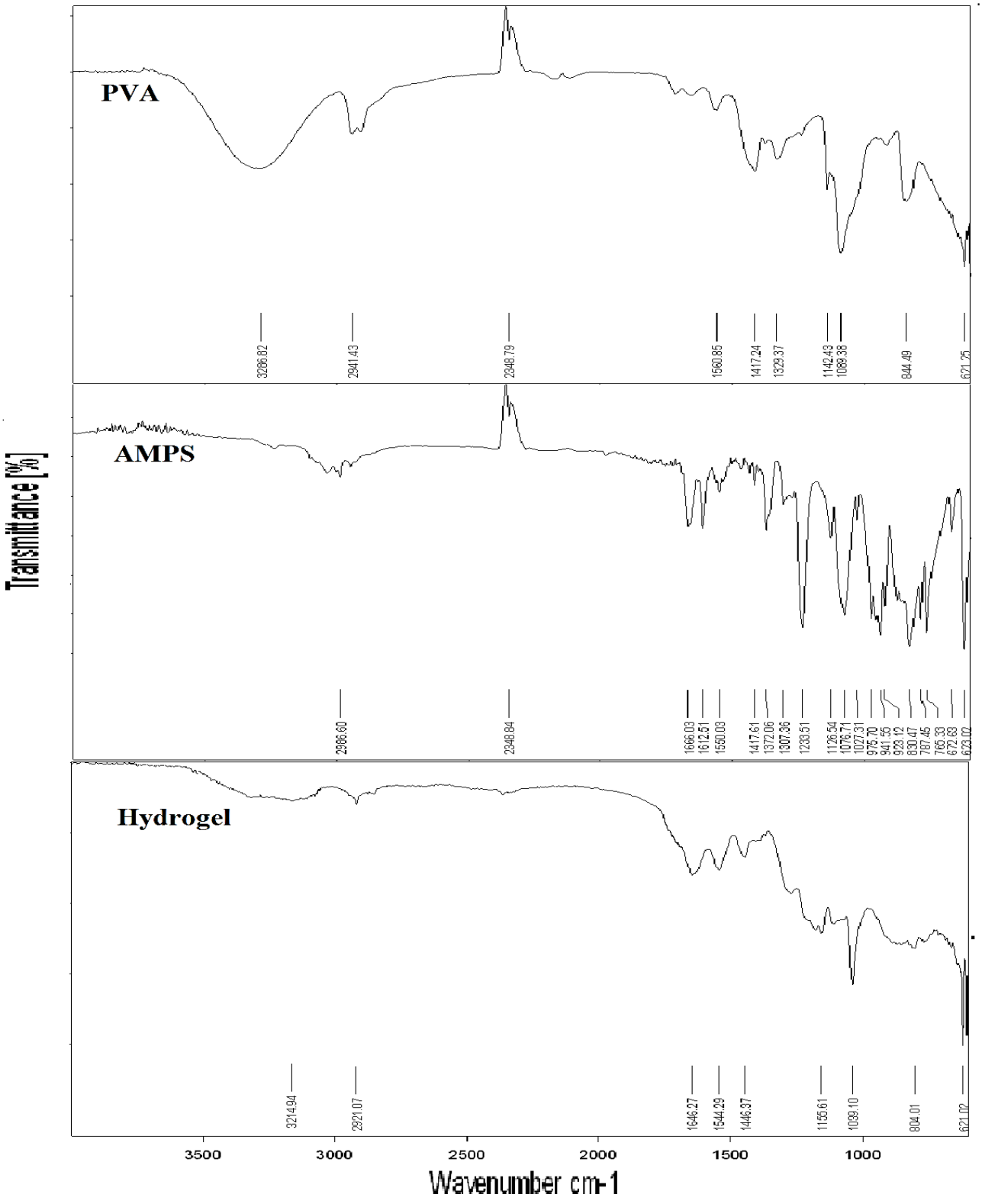

Figure 2. FT-IR spectra of PVA, AMPS and PVA-co-AMPS hydrogel

5.4.2 SEM

For the determination of microstructure and surface morphologies of formed graft copolymer scanning electron microscopy was performed. The SEM images of hydrogels were taken at different magnifications of 800 X and 5000 X in order to visualize their surface roughness and presence of pores. Figure 3 (A and B), describes that the hydrogel formulation SP5 prepared under influence of relatively lower radiation dose had a highly porous surface; indicating its ability to absorb higher quantity of water and entrap more solute particles. In comparison, the hydrogel SP3 comprising of same amounts of polymer, monomer and crosslinking agent as that of SP5 but formed by higher doses of radiation had less porous surface. The SP3 hydrogel formulations had cracked surface due to bursting effect at higher electric power. Exposure to microwave radiations leads to formation of highly porous surface because of their uniformly rapid and instantaneous penetration.[272] It was determined by Zhao *et al.*[72] who compared the SEM images of hydrogels prepared by microwave radiations with hydrogels prepared by conventional water bath method.

The presence of surface roughness and porous structure are directly related to swelling tendency of polymeric network. It indicates the loose crosslinking among PVA and AMPS in hydrogel formulation SP5. In hydrogel formulation SP5, there was lesser crosslinking among the polymer and monomer due to its exposure to low intensity of radiation (180 W). Moreover, the crosslinking reaction was initiated by low concentration of initiator (0.15% W/W) under the effect of microwave radiations.

In comparison to SEM image of SP5 hydrogel, SP3 hydrogel formulation prepared by receiving higher radiation doses at electric power of 300 W had dense and tight structure. The surface roughness was reduced with lesser porous structure, having lower tendency to absorb water and swell in aqueous fluids. This was due to greater crosslinking among the components due to intense radiations and relatively higher amounts of potassium persulfate (0.25% W/W) as initiator. Moreover, the exposure of hydrogels for same time period at electric power 300 W led to formations of cracks as illustrated in figure 3 (C and D).

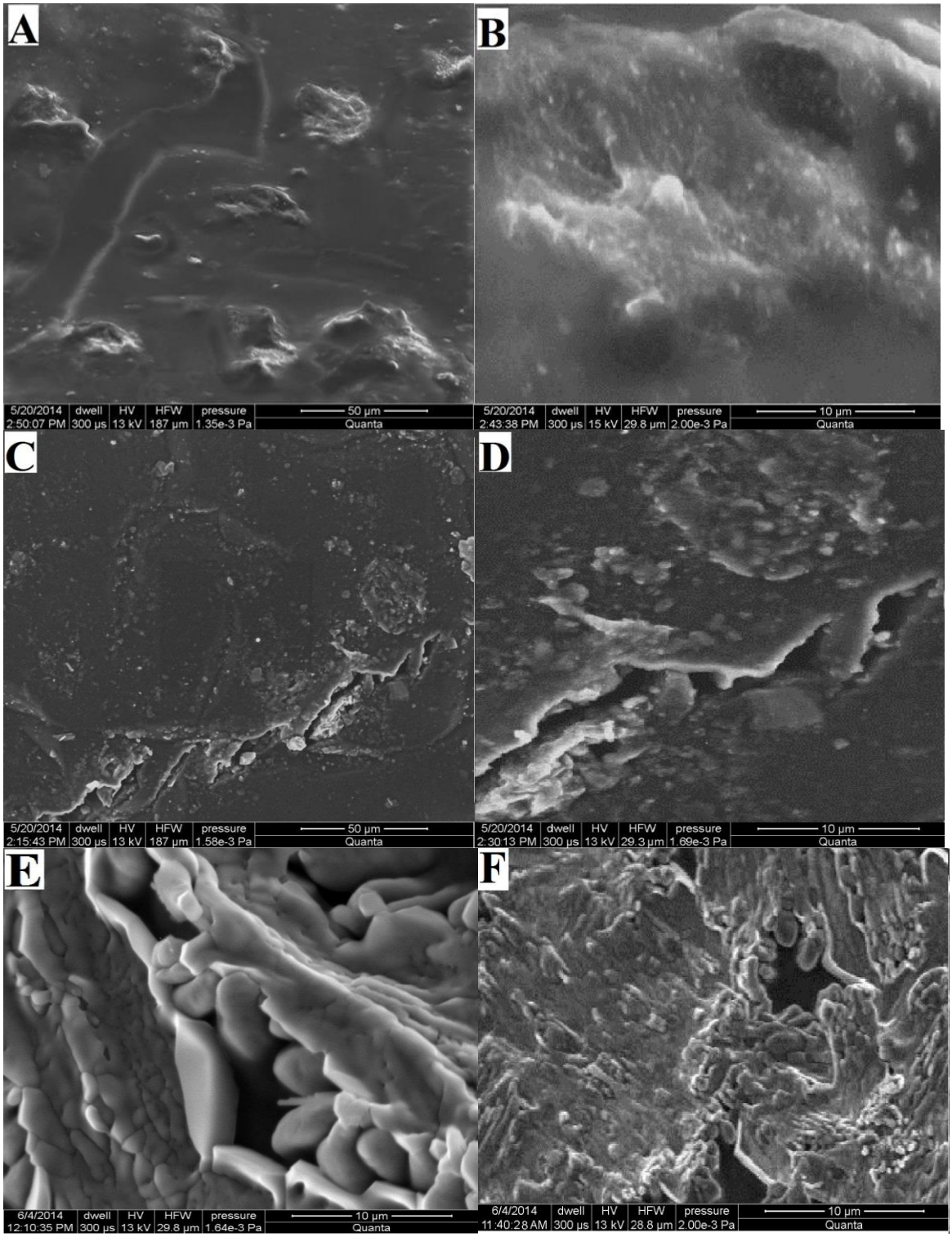

Figure 3. SEM images at different magnifications A) SP5 at 800x, B) SP5 at 5000x, C) SP3 at 800x, D) SP3 at 5000x, E) Drug loaded SP5 at 5000x, F) Drug loaded SP3 at 2500x

Hence, it was estimated that SP5 formulation had higher swelling tendency and drug loading capacity as compared to SP3 formulation. These results were in accordance to determination of grafting among the polymers by Huacai *et al.*[283] and Wan *et al.*[274] They prepared the hydrogels were under different powers of microwaves and their moisture absorption was evaluated, which was higher in hydrogels receiving lower doses of microwaves. The effect of initiator on grafting determined by them was also indicative of porosity and swelling behavior.

The entrapment of solute particles in hydrogels depends upon surface roughness as well as porosity is related with quantity of drug loaded. It could be clearly seen from SEM images shown in figure 3 E and 3 F for drug loaded hydrogel SP5 and SP3 respectively, that higher amount of drug could be loaded in SP5 due to its more porous structure.

5.4.3 XRD

The diffraction patterns of hydrogels loaded with drug were compared with pure drug. X-ray diffractograms of pure Captopril and drug-loaded Hydrogels are presented in Figure 4. The XRD scan of plain Captopril had shown characteristic sharp and intense peaks between 0° and 50° (2θ) due to its crystalline nature as shown in Figure 3 diffraction pattern (b). The appearance of a sharp peak at 2 θ= 27.79° is the characteristic of captopril.

In comparison to diffractogram of pure captopril, the Captopril loaded formulations had low intensity and dense peaks. The sharp peak of drug were significantly reduced suggesting the amorphous distribution of drug into polymeric network, as it can be observed from figure 4 diffractogram (a).

Captopril is a crystalline drug and the presence of intense peaks in diffractrogram ‘b’ was due to crystallinity of the Captopril. On the other hand, the hydrogels formed by crosslinking of polymers usually develop an amorphous structure with denser peaks. After loading of drug into PVA-co-AMPS hydrogel, the captopril was entrapped and dispersed in hydrogel formulation. The characteristic sharp peak at 2 θ= 27.79° in pure captopril was much reduced and broadened in drug loaded hydrogel as could be clearly observed from diffractograms a and b.

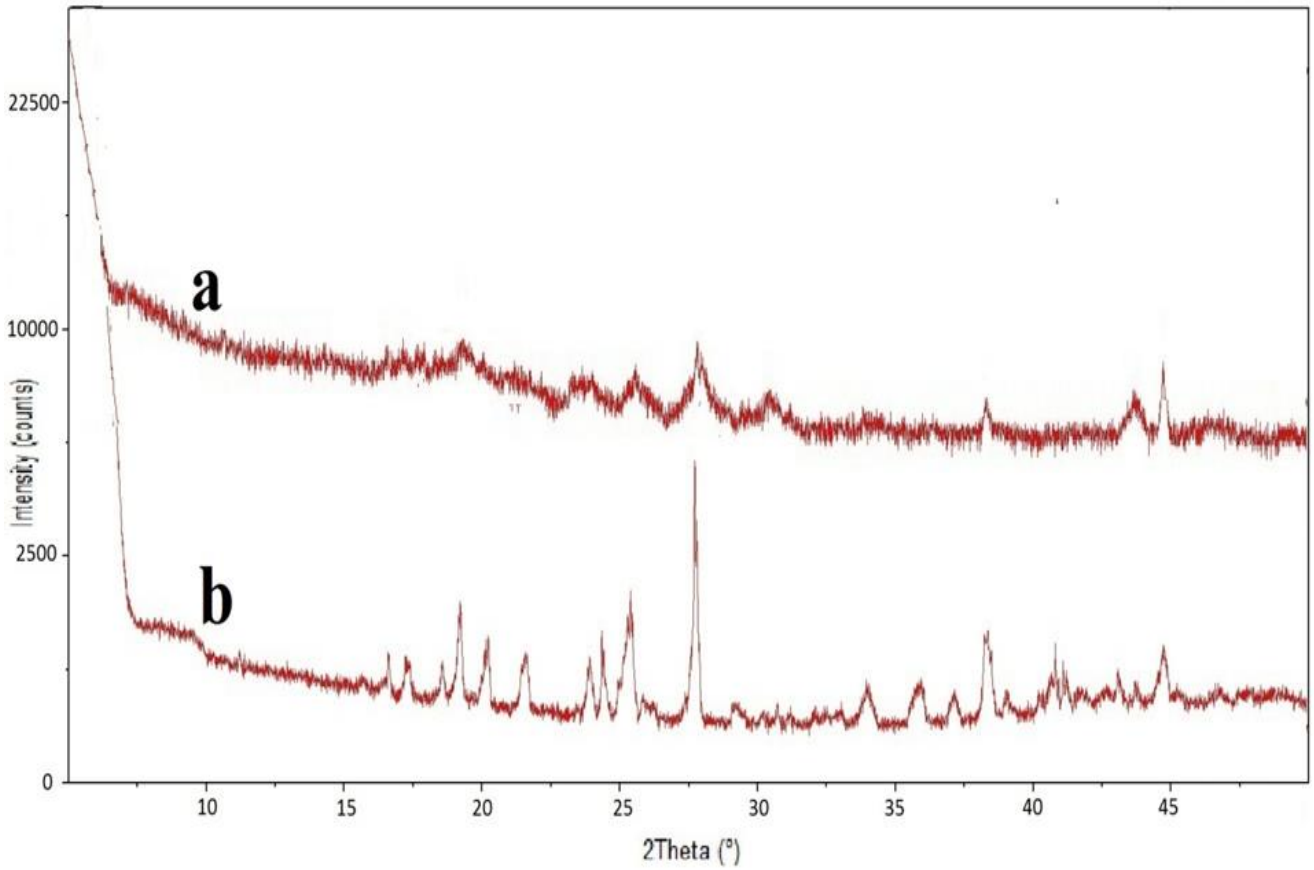

Figure 4. X-Ray Diffraction patterns of Drug loaded hydrogel SP5 (a) and pure Captopril(b)

Therefore, the intensity and sharpness of peaks was reduced in captopril loaded hydrogel, only indicated the presence of entrapped drug into polymeric networks of hydrogel formulation. The resultant evaluation on basis of XRD analysis was in relevance with observations noticed by Giri *et al.*,[282] where diltiazem hydrochloride was loaded into cross-linked biodegradable IPN hydrogel beads of pectin and modified xanthan gum. Similar diffraction patterns were noted for pure drug and drug loaded hydrogels.

5.4.4 Thermal analysis

In order to determine the thermal stability and confirmation of crosslinking, thermal analysis was performed using Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Figure 5 illustrates the TGA thermograms of PVA, AMPS and formed polymeric network. Being monomer, AMPS was less stable at higher tempratures, it was decomposed completely at 243°C. PVA shows stability upto 227°C, till that temperature there was only 10% weight loss, which was

due to loss of water molecules and it was decomposed at about 300°C. At 325°C, weight loss observed was 70% that degrades complete structure of the polymer. In comparison, thermogram of hydrogel shows a gradual decrement in stability, indicating its enhanced thermal stability. At 300°C, there was only 25% weight loss and at 489°C still 34% was remaining mass, whereas PVA was 0.07% at 477°C. Such different stages of thermal degradation in TGA analysis were also evaluated by by Huacai *et al.*[283] and Bao *et al.*[95]

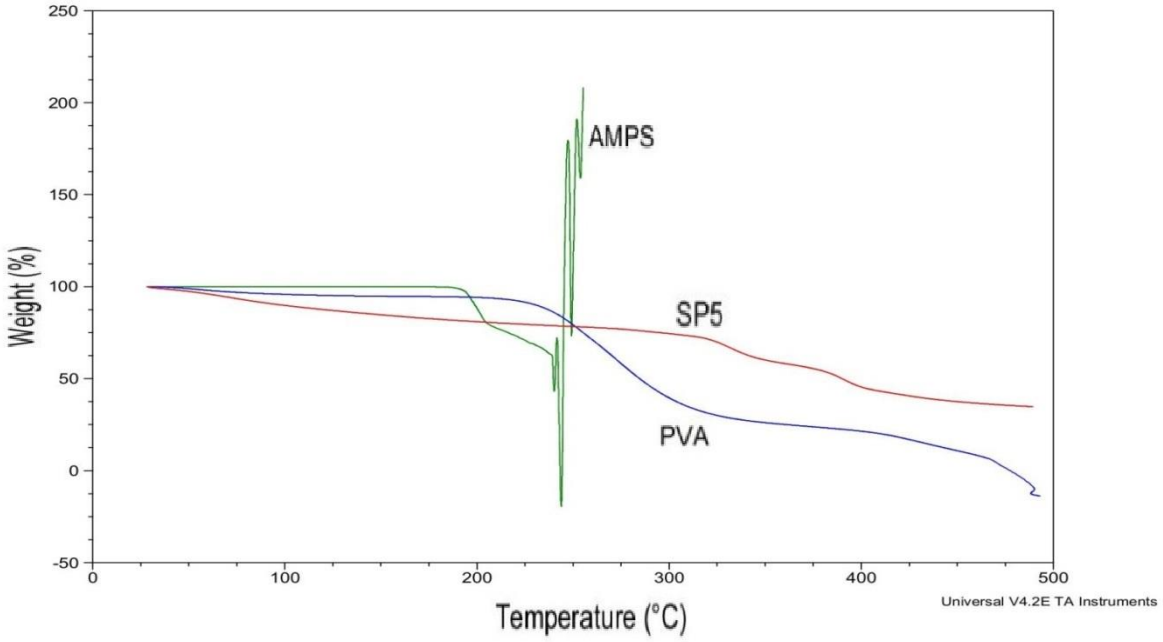

Figure 5. TGA analysis of AMPS, PVA and hydrogel formulation SP5

From the TGA thermograms of individual components (PVA and AMPS) and formed hydrogel, it could be clearly seen that the developed hydrogel formulation SP5 had sufficiently strong crosslinked structure to retain itself for longer time periods. Therefore, maintenance of stability in the polymeric network at high temperature in comparison to uncrosslinked polymer and monomer was confirmed by TGA.

The alterations in heat capacity as well as enthalpy changes were measured using Differential Scanning Calorimetry (DSC). Figure 6a and 6b show the DSC-TGA thermograms of hydrogel and comparative DSC thermograms of drug, drug free hydrogel and drug loaded hydrogel. The DSC endothermic peaks of cross-linked polymeric network were in accordance with TGA thermal patterns as shown in figure 6a. Both TGA and DSC

thermograms indicates that the deterioration of PVA-co-AMPS started nearly at temperature 250°C.

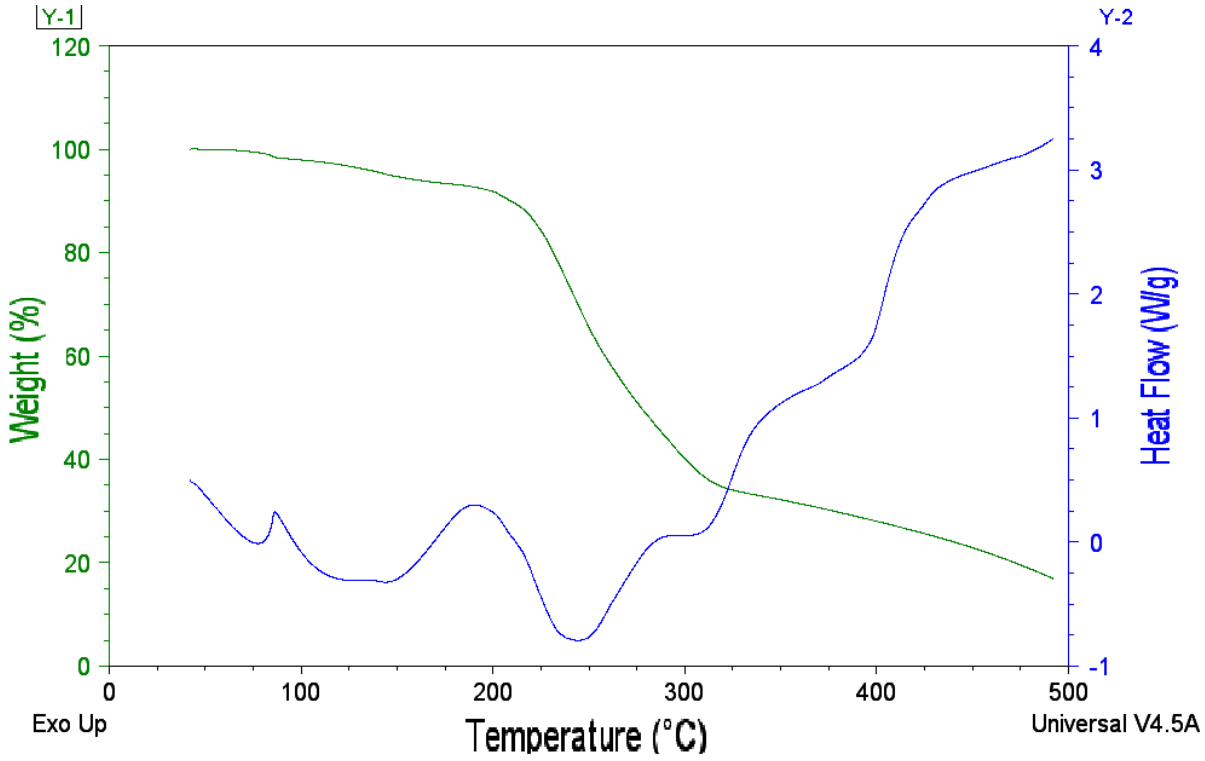

Figure 6a. DSC-TGA thermogram of PVA-co-AMPS Hydrogel

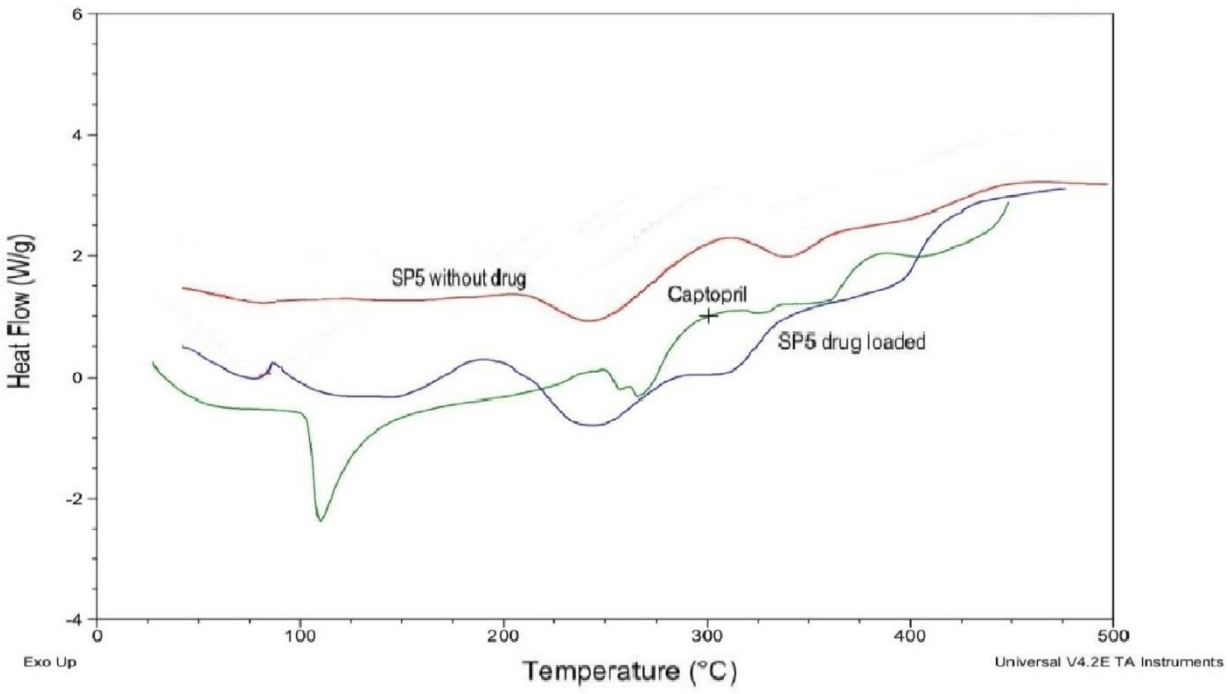

Figure 6b. DSC analysis of SP5 without drug, captopril and Drug loaded SP5

Differential Scanning Calorimetry (DSC) is a well-known and established technique adopted for quantitative assessment of physicochemical variations in heat capacity of crystalline drugs, when loaded into hydrogels. The thermal behavior of pure captopril, drug free and drug loaded SP5 hydrogel was characterized using DSC, as shown in Figure 6b. The disappearance of characteristic peaks and appearance of other peaks suggests some sort of interaction of drug with polymers. In figure 6b, the thermogram of pure captopril, the appearance of sharp peak at 106°C indicated its melting point. This peak of drug was not shown in DSC thermogram of the drug loaded formulation. A broad peak from 97°C to 160°C was appeared in thermogram of drug loaded hydrogel. It suggests that captopril is molecularly dispersed in different hydrogel matrix without changing its thermal behavior. Both formulations, without drug and drug loaded had shown major endothermic peak at about 240°C, which illustrated the stability of formulation after drug loading without any marked alteration in thermal stability pattern. These observations are relevant to DSC analysis of aceclofenac sodium loaded hydrogels evaluated by Manjanna *et al.*[281]

5.4.5 Swelling Study

The swelling ratios 'S' and percent swelling equilibrium (%ES) were calculated by performing the swelling studies at pH 2 and 7.4. The pH 2 represents the pH of gastric medium in the stomach and pH 7.4, the intestinal pH. The current work was aimed to develop a gastroretentive hydrogel formulation; therefore, it must exhibit higher swelling rates at acidic pH. Greater swelling ratios were observed at pH 2 as compared to swelling at pH 7.4 in a same manner as determined by Gupta *et al.*[340] However, it was varying from their work due to constitution of polymeric network. Various other factors were affecting the swelling/water absorption into the prepared hydrogel formulations are discussed as given below:

Effect of microwave radiation

In order to provide a proper swelling, the polymers must be sufficiently crosslinked, so that the obtained material should not dissolve in aqueous medium. The crosslinking density in hydrogels depends upon the power of microwaves received. Figure 7 illustrates the swelling ratios of SP1, SP2, SP3 and SP4 hydrogels treated with different intensities of microwave radiation. These observations are in correspondence to morphological characterizations performed by scanning electron microscopy. The comparison of swelling capability of these formulations was observed at lower pH 2 and higher pH 7.4.

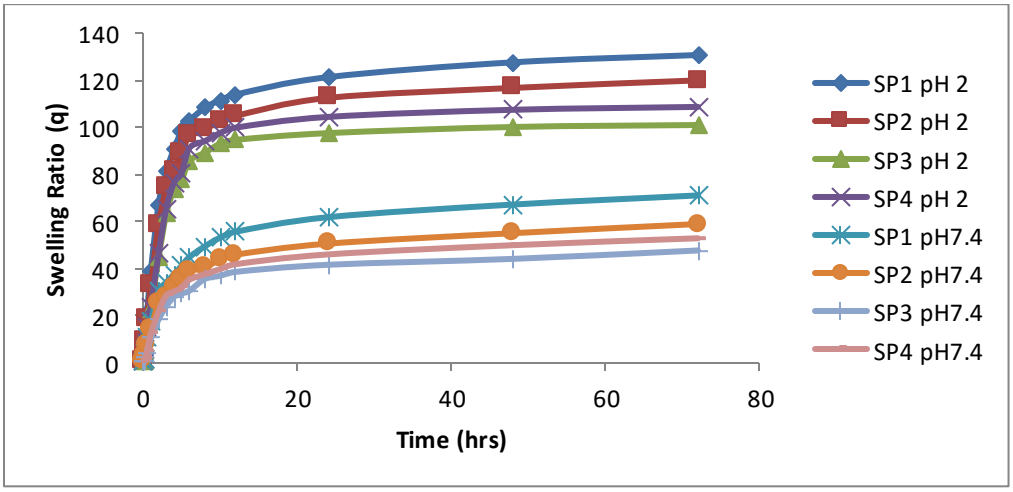

Figure 7. Comparative swelling ratios of Formulations SP1, SP2, SP3 and SP4 at pH 2 and 7.4

The hydrogels treated at 100W were possessing higher swelling than one exposed at 180W, both containing same concentration of crosslinker, PVA and AMPS. The hydrogels exposed further to increased radiation dose i.e. at 300W exhibited low swelling, due to greater crosslinking density among polymers. The high doses above 180W also cause the destruction of crosslinked structure, that provide an irregular and lesser swelling than other treated at lower frequency of microwaves.

Hydrogel formulation SP2 (prepared at 180 W) was considered more suitable due to sufficient strength to maintain its disc integrity while swelling, so that it could remain stable in the stomach for longer periods of time. However, formulation SP1 had shown more swelling behavior but lesser mechanical strength due to low crosslinked density. SP3 and SP4 hydrogel formulations treated at 300 W were showing lesser swelling ability. Figure 8 illustrates the effect of microwave radiation on percent equilibrium swelling.

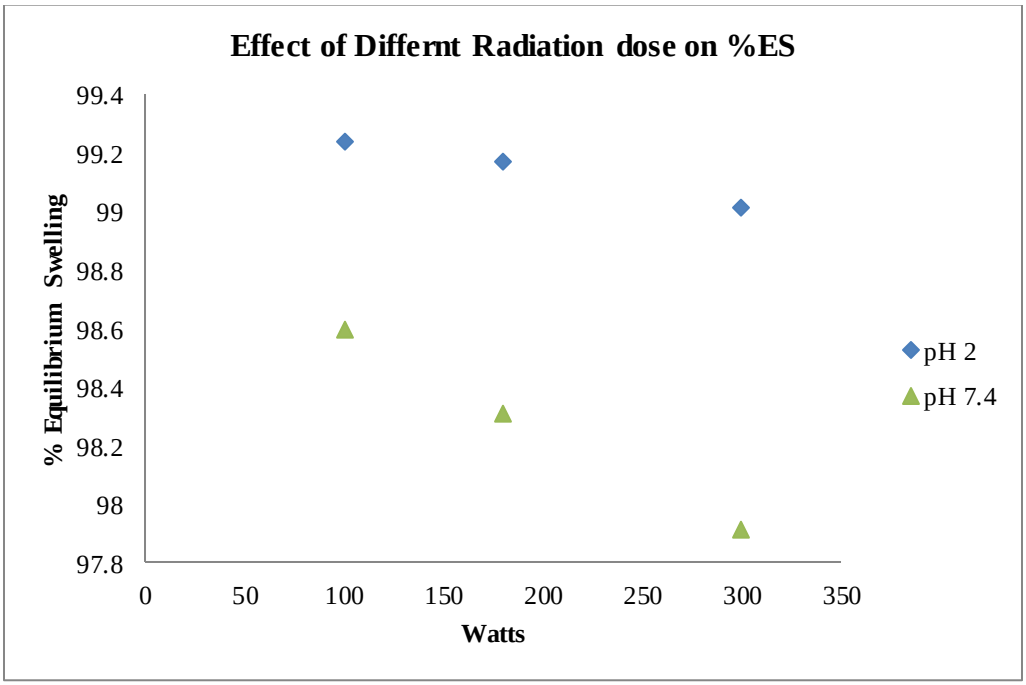

Figure 8. Effect of microwave radiation dose on Percent Equilibrium Swelling

Figure 8 shows that by increasing the intensity of radiation exposure to hydrogel formulations led to decrement in percent equilibrium swelling. Thus, it was observed that microwave radiations due to their ability to initiate the crosslinking reactions effect crosslinking density and ultimately the swelling of polymeric network. These observations

were related to earlier findings of Singh *et al.*,[267] and Wan *et al.*[274] where hydrogels were treated with different intensities of microwave radiations and there moisture absorption properties were determined, which were inverse of percent grafting. Due to higher grafting, the polymeric networks have more crosslinking density that ultimately reduces swelling ratio.

Effect of Initiator concentration

Due to ability of microwave radiations to initiate the reaction for crosslinking of polymers, lower concentrations of initiator were required. However, increasing the initiator concentration increases the crosslinking among polymers and consequently reduces the swelling characteristics as shown in figure 9 and 10. Figure 9 illustrates the comparative swelling ratios of formulations SP2, SP5 and SP6 at pH 2 and 7.4. These formulations were exposed to same radiation dose of 180W as well as comprising of same ratios of all components except initiator concentration.

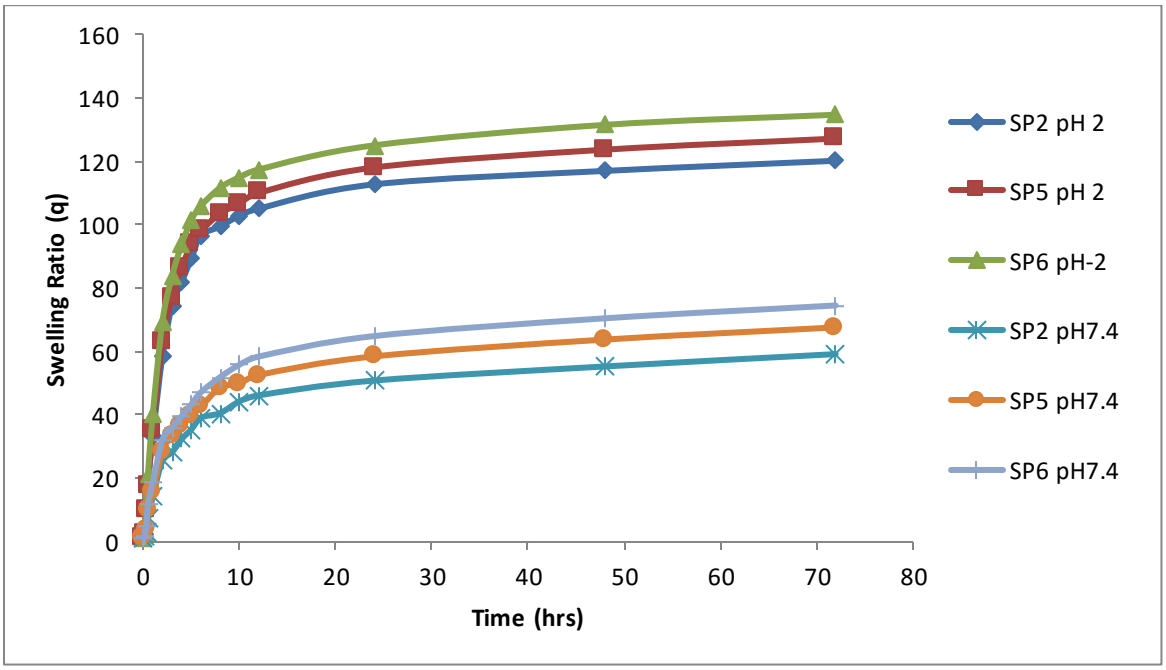

Figure 9. Comparative swelling ratios of Formulations SP2, SP5 and SP6 at pH 2 and 7.4

The hydrogel formulation SP2 containing higher initiator concentration (0.25% W/W) had shown relatively low swelling ratio. Swelling of SP5 hydrogel was higher than SP2 as SP5

hydrogel had lesser concentration of initiator (0.15 % W/W). Moreover, highest swelling ratio of SP6 hydrogel was observed among other (SP2 and SP5) as it had lesser amount of initiator (0.05% W/W). Figure 10 is showing the effect of initiator concentration on Percent Equilibrium Swelling. It illustrates that increasing the concentration of initiator decreases the percent equilibrium swelling.

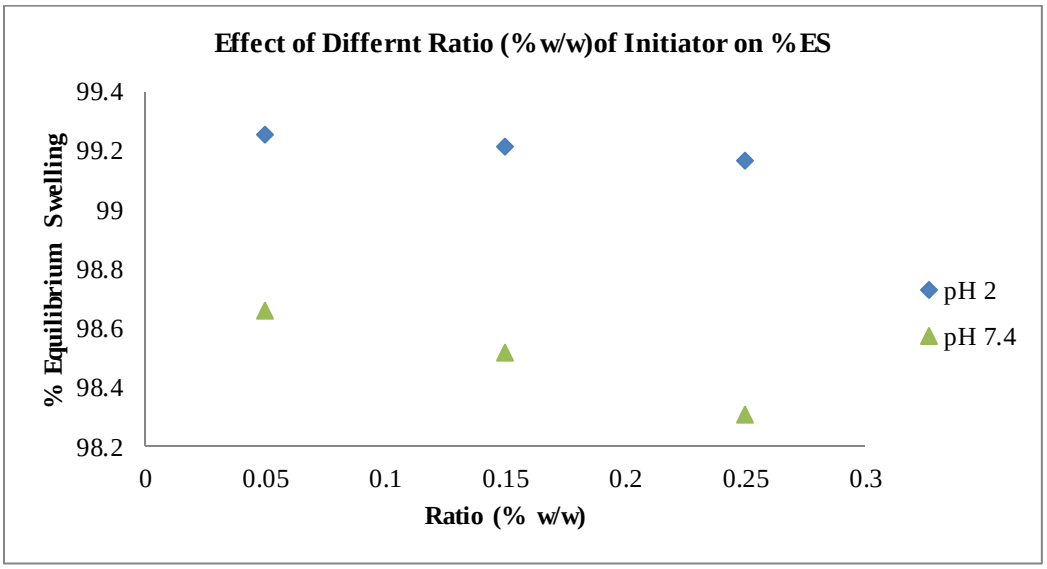

Figure 10. Effect of initiator concentration on Percent Equilibrium Swelling

The resultant reduction in swelling tendency with increasing initiator concentration were confirmed earlier by Cheng *et al.*[268] and Wan *et al.*[274] They observed an increment in water absorption capacity of hydrogels by using an optimum initiator concentration, under influence of microwave radiations. Above, the optimum amounts of initiator the water absorption characteristics were showing remarkably decreased.

Effect of AMPS Concentration

The effect of monomer concentration on swelling capability was determined that how the quantity of AMPS affects the water absorption characteristics of superporous hydrogel formulations. SP5, SP7 and SP8 formulations containing different amounts of AMPS but same concentration of polymer (PVA- 1% W/W), crosslinker (MBA- 1 mol % of monomer concentration), initiator (KPS- 0.15%W/W) and radiation dose were compared in terms of swelling ratios as illustrated in figure 11.

It was observed that by increasing the amount of monomer AMPS in formulation, there was increment in swelling behavior. Variation in swelling ratio among these hydrogel formulations (SP5, SP7 and SP8) was due to higher quantity of AMPS.

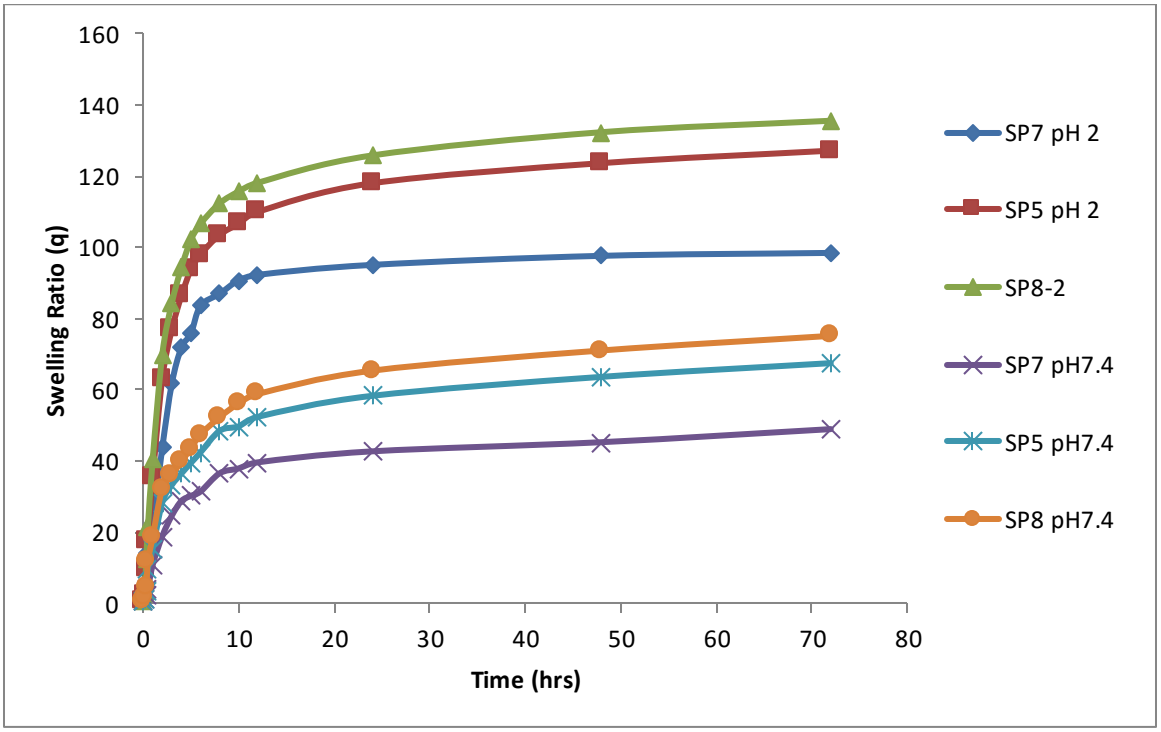

Figure 11. Comparative swelling ratios of Formulations SP5, SP7 and SP8 at pH 2 and 7.4

AMPS cause the availability of more sulfonate groups, which increase the hydrophilic characteristics in polymeric network. At acidic pH, a remarkable ability of water absorption was noticed because of high ionization of functional groups in AMPS. In addition to ionization of sulfonic groups, the hydrogen bonds are also reduced that consequently increased the swelling power of the formed superporous hydrogel. The hydrogel formulation SP8 possess high quantity of AMPS (30 % W/W) had shown greater swellability as shown in figure 11. In SP5 formulation, AMPS was lesser than SP8, therefore it had shown lesser comparative swelling ratio, but it was higher in comparison to SP7 containing 20 % W/W of hydrogel formulation. Increment in swelling tendency with increasing AMPS content is corresponding to swelling study performed on AMPS based hydrogel by Qudah *et al.*[239] On the other hand it was varying from the observations made by Bao *et al.*,[95] where AMPS was cross-linked with a carboxymethylcellulose in the presence of acrylic acid and acrylamide,

while in the present work, AMPS was only monomer grafted with polymer. Therefore, the swelling behavior is dependent upon the amount of AMPS. Figure 12 is showing the effect on AMPS concentration on percent equilibrium swelling.

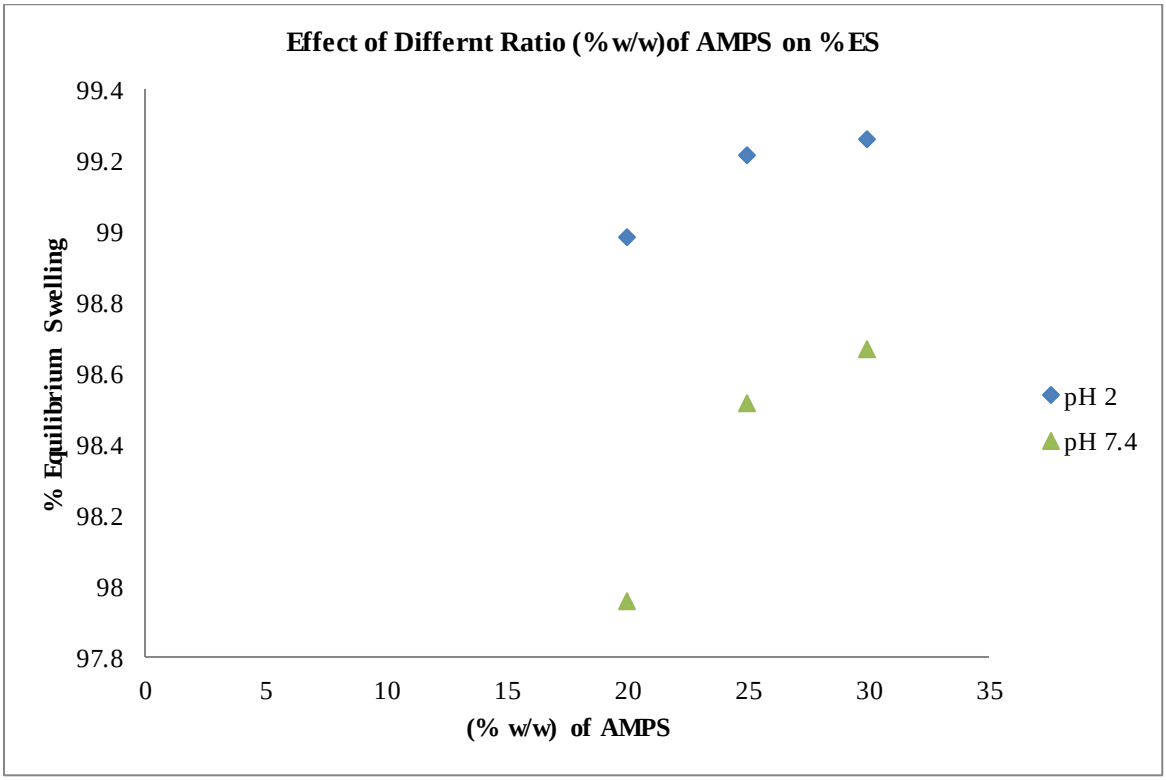

Figure 12. Effect of AMPS concentrationon Percent Equilibrium Swelling

It could be observed from the figure 12 that by enhancing the amount of AMPS had made an increment in percent equilibrium swelling of hydrogel formulations. AMPS created the availability of ionizable sulfonate groups in the prepared superporous polymeric network, which ultimately led to enhancement of their swelling characteristics.

Effect of Polymer concentration

The effect of polymer concentration on swelling behavior was confirmed by comparing three hydrogel formulations containing same quantities of monomer, crosslinker, initiator and radiation dose but different amounts of polymer. Hydrogel formulations SP5, SP9 and SP10 were evaluated for their swelling properties as shown in figure 13.

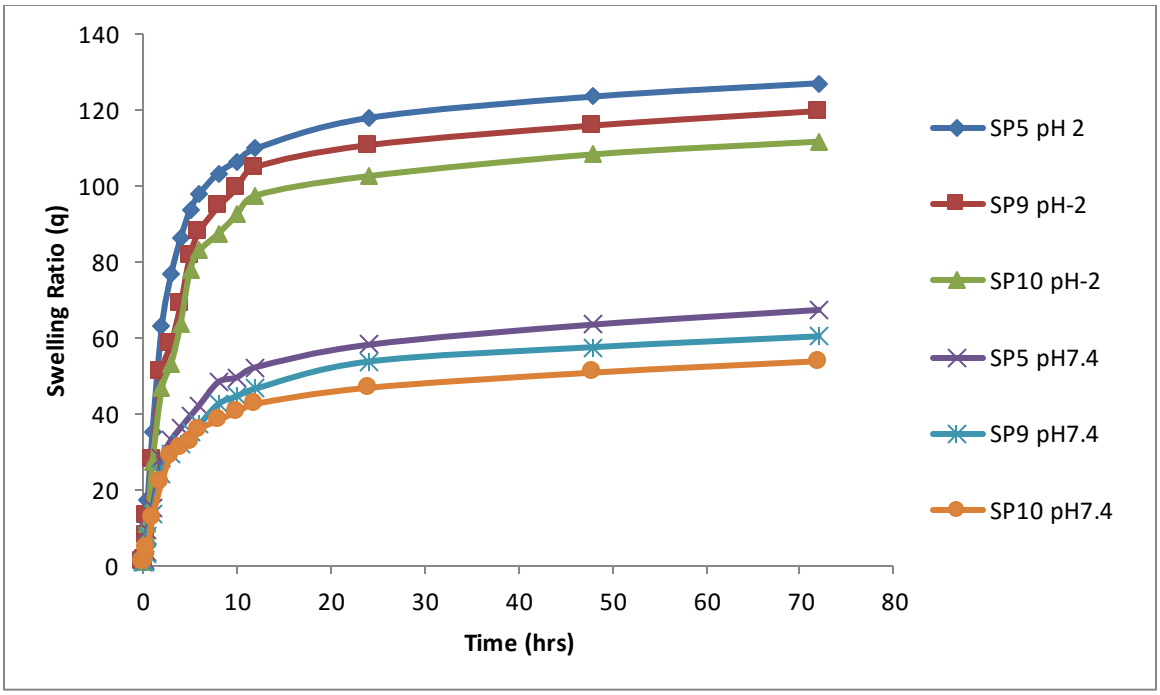

Figure 13. Comparative swelling ratios of Formulations SP5, SP7 and SP8 at pH 2 and 7.4

SP5 formulation had lower concentration of PVA (1% W/W) in comparison to SP9 and SP10 with PVA concentration 2% and 3%, respectively. A higher swelling ratio of SP5 hydrogel was noted than other formulations (SP9 and SP10). SP10 formulation, because of high polymer amount had lower swelling than both SP5 and SP9. Figure 14 depicts the effect of polymer concentration on percent equilibrium swelling of hydrogels.

These observations are in correspondence to swelling study by Tyliszczak[211] and Minhas *et al.*[339] where a decrement in swelling ability of hydrogels was observed by increasing the amount of polyvinyl alcohol. However, the results of swelling evaluation were differing from work of Hosseinzadeh,[347] who observed increase in swelling by increasing PVA concentration from 1.2 to 2.4 wt% in PVA-based hydrogels. By increasing further amount of PVA created a decrement in absorption characteristics, due to increasing viscosity of reaction medium that restricts the movements of PVA chains. In comparison, in our case, a decrease in swelling ratio was noted by increasing the amount of PVA from 1 to 2 wt% in AMPS-based hydrgels.

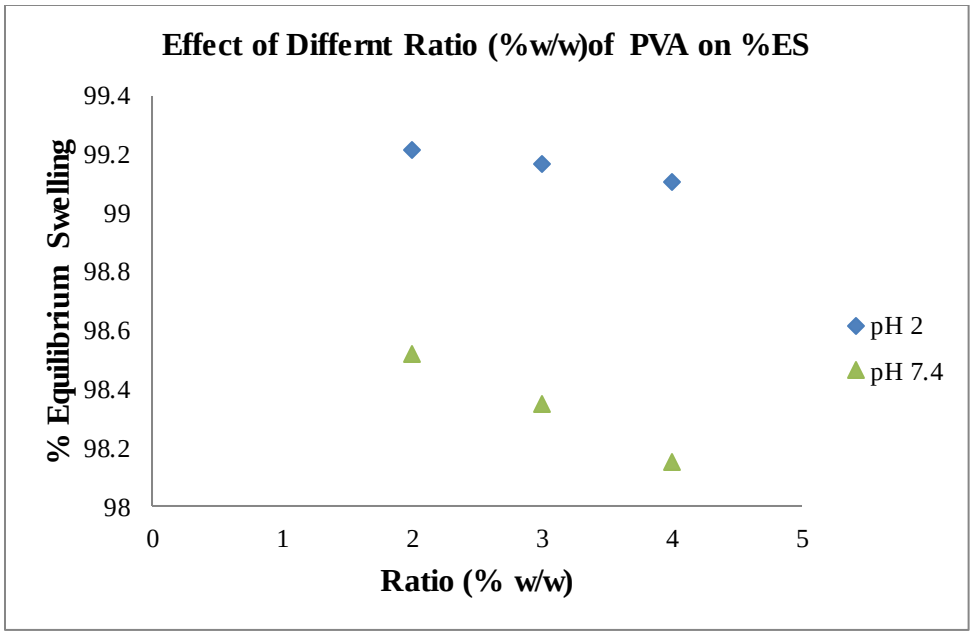

Figure 14. Effect of PVA concentrations on Percent Equilibrium Swelling

Polvinyl alcohol PVA imparts mechamical strength to the polymeric system and possesses water absorption characteristics, but its swelling capability is low in comparison to AMPS. Hence, by increasing the quantity of PVA in hydrogel formulation, causes a reduction in swelling behavior as it could be observed from figure 13 and 14.

5.4.6 *In-vitro* drug release studies

After loading of captopril into the prepared superporous hydrogel formulations, the drug release study was performed at pH 2 and pH 7.4 as shown in table 2. The drug release study at mentioned pH was performed for a period of 24 hours, where USP phosphate buffer was used as dissolution medium. All the formulations were subjected to drug loading and the release of entrapped captopril was evaluated. The hydrogel discs possessing higher swelling ratio were able to entrap higher quantity of drug. The amount of drug release is according to swelling characteristics, where drug loading is directly related to water retention capability of polymeric network. The hydrogels which exhibited higher swelling ratio entrapped more amount of captopril.

Table 2. Amount of Captopril loaded and percentage of drug released at pH 2 and pH 7.4

sample code	Amount of captopril loaded (mg) per 0.3 gram of dry hydrogel discs	% release of captopril	
		pH 2	pH 7.4
SP1	202.5	98.08	72.32
SP2	186.8	85.11	54.26
SP3	169.8	69	47.45
SP4	173.8	73.13	49.56
SP5	189.8	93.21	66.95
SP6	194.7	95.93	70.02
SP7	156.78	56.95	33.73
SP8	199.8	97.38	68.23
SP9	179.67	82.27	55.66
SP10	171.36	73.26	48.85

Hydrogel formulations SP1, SP6 and SP8 were loaded with higher quantity of drug and ultimately had shown high amounts of drug release at both lower and higher pH (2 and & 7.4). Moreover, drug release was higher at acidic pH as compared to pH 7.4. This was due to swelling characteristics of AMPS, which is not much dependent on pH of medium. However, being an AMPS-based hydrogel it exhibited swelling to greater extent in low acidic pH as compared to neutral or higher alkaline pH.

Figure 15 presents a comparison of three hydrogel formulations SP1, SP5 and SP10. Among them, the SP1 hydrogel, the components were loosely crosslinked due to exposure at low dose of microwave radiation, therefore it entrapped and released higher quantities of captopril. On the other hand, formulation SP10 had shown lower swelling ratio due to higher PVA concentration, hence lower drug loading and drug release was observed. Thus, relatively more amount of drug was loaded and ultimately released in formulations exhibiting more swelling power. The drug release kinetics of prepared superabsorbent hydrogels was determined by different kinetic models mentioned in table 3. The kinetic models used were

Zero order kinetics, First order kinetics, Higuchi model, Korsmayer-Peppas model and Wilbull model.

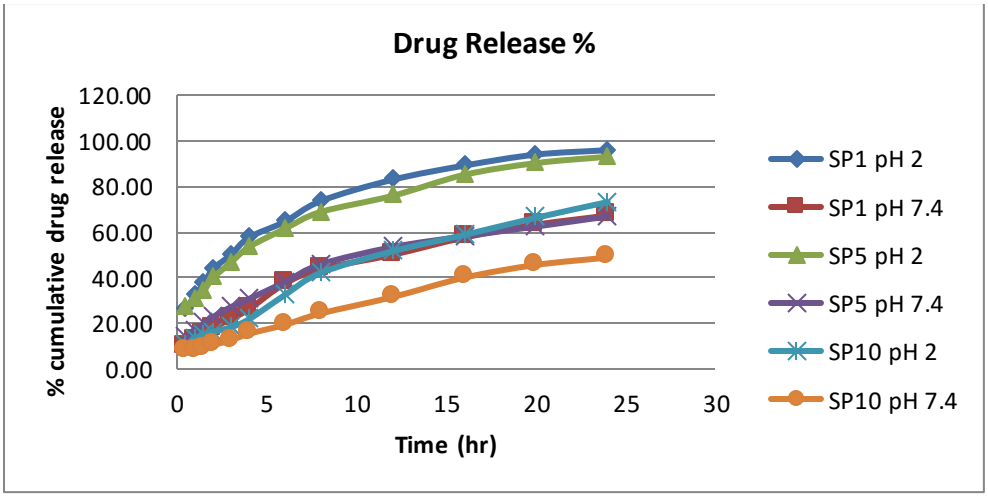

Figure 15. Captopril released up to 24 h from PVAco-AMPS hydrogels (SP1, SP5, and SP10) in dissolution media of pH 2 and pH 7.4

Table 3. Determination of coefficient (R^2), K and release exponent of various drug release kinetic models

Sample code	pH	Zero order kinetics		First order kinetics		Higuchi model		Korsmeyer-Peppas model			Wilbull model
		R^2	K	R^2	K	R^2	K	R^2	K	n	R^2
SP1	2	0.8745	5.6976	0.2887	0.3167	0.9716	26.424	0.9853	46.38	0.17	0.7897
	7.4	0.9342	3.5779	0.4773	0.2569	0.9907	13.688	0.9751	17.0869	0.37	0.8113
SP2	2	0.9523	4.2344	0.3654	0.2920	0.9976	19.968	0.9978	26.9387	0.33	0.8703
	7.4	0.959	2.9744	0.4552	0.2466	0.9914	11.024	0.9855	7.7882	0.7212	0.8429
SP3	2	0.7901	5.2216	0.2508	0.3005	0.9136	20.596	0.9864	28.0953	0.504	0.722
	7.4	0.9057	2.7629	0.4807	0.2357	0.9834	10.309	0.9841	15.3971	0.644	0.7661
SP4	2	0.8196	5.3027	0.2702	0.3015	0.9388	21.272	0.9875	33.8937	0.2203	0.7465
	7.4	0.888	2.8506	0.425	0.2471	0.9745	11.571	0.9837	16.3121	0.3081	0.7492
SP5	2	0.9027	4.8442	0.3018	0.3106	0.9833	24.761	0.9923	40.3292	0.2164	0.8114
	7.4	0.9268	3.5394	0.4	0.2699	0.9908	15.37	0.9871	20.0108	0.35	0.829
SP6	2	0.8745	5.9259	0.2866	0.3198	0.9716	27.483	0.9853	48.2390	0.1749	0.7898
	7.4	0.9342	3.7212	0.4733	0.2600	0.9907	14.237	0.9751	17.7714	0.3792	0.8115
SP7	2	0.9765	2.7670	0.5472	0.2335	0.9962	10.381	0.9978	9.2038	0.568	0.8643
	7.4	0.9944	1.3716	0.6247	0.1916	0.9738	5.998	0.9861	4.6877	0.647	0.9277
SP8	2	0.8745	5.7746	0.288	0.3177	0.9716	28.781	0.9853	47.0076	0.1749	0.7898
	7.4	0.9342	3.6262	0.4759	0.2579	0.9907	13.873	0.9751	17.3178	0.3792	0.8114
SP9	2	0.9681	4.0971	0.4882	0.2671	0.997	15.574	0.9954	15.8369	0.4907	0.8594
	7.4	0.9917	1.9592	0.5609	0.2274	0.9716	9.843	0.991	7.3925	0.6717	0.9289
SP10	2	0.9692	3.2586	0.5306	0.2497	0.9865	13.233	0.9814	12.2555	0.54	0.8774
	7.4	0.9879	1.9694	0.6073	0.2160	0.9826	8.474	0.9977	8.7381	0.48	0.8986

The results calculated in terms of values of release coefficient and release exponent from PVA-co-AMPS copolymer containing different components, polymer concentration, initiator concentration and radiation dose. The mechanism of drug release was indicated by the values of n i.e. release exponent as presented in table 3.

The formulations were best fitted into the above mentioned kinetic models when values of R were near to '1'. There was variation in value of 'n' among the formulations prepared. The SP7 formulation containing lower monomer concentration, SP9 and SP10 hydrogel formulations with higher amounts of polymer were exhibiting non-fickian diffusion mechanism of drug release with value of n greater than or equal to 0.45 but lesser than 1. Moreover, SP3 formulation receiving higher dose of radiation (300 W) and higher initiator concentration were also following non-fickian diffusion. Other formulations with higher amounts of monomer and lesser polymer concentration were following fickian diffusion with value of 'n' less than 0.45.

Conclusion

It was concluded from the discussion, that super-porous hydrogels were prepared successfully under influence of microwave radiations. Using low concentrations of initiator could successfully develop polymeric network of polyvinyl alcohol and AMPS by microwave radiations. The formed hydrogel had ability to swell excessively at acidic pH and it could be retained in the stomach, releasing captopril at low pH for longer durations of time. Therefore, the synthesized copolymer could be a promising candidate as carrier for captopril in treating hypertension.

Chapter no.6

In-vivo Evaluation of hydrogel formulations for Controlled Release Drug Delivery of Captopril

Abstract:

A specific high performance liquid chromatography–ultravoilet spectrometric (HPLC-UV) assay was developed for the determination of captopril in plasma. It was conducted on prepacked Hypersil C8 column at room temperature using Phosphate buffer: acetonitrile (75:25 v/v) as a mobile phase, pH adjusted at 2.8 with *o*-phosphoric acid and at a flow rate of 1.0 ml/min, while UV detection was performed at 205 nm. The retention time was 6.5 min for captopril. The liquid-liquid extraction method was used for the detection of captopril. Dithiothreitol was added in extraction medium in order to stabilize the captopril extracted from plasma. Standard curve was linear in captopril concentration ranging from 50 to 2000 ng/ml. The method had a suitable sensitivity to detect drug at low concentrations due to its lower limit of quantification (LLOQ) value noted as 50ng/ml. Intra-batch as well as inter-batch precision and accuracy measured had shown good results. The extraction efficiency of captopril was ranging from 95 to 99.9 %. The method developed was applied for the pharmacokinetic study in 24 rabbits for evaluation of pharmacokinetic parameters of captopril. The method was simple, rapid, reliable, specific and sensitive with ability to determine drug plasma concentrations from rabbits for longer duration.

6.1 Introduction

Angiotensin converting enzyme (ACE) inhibitors are commonly used for treatment of heart diseases such as hypertension and heart failure. Among them, captopril, is an orally active potent ACE inhibitor and widely accepted due to its antihypertensive action attained within 45 min to 1 hour after oral administration.[348-351] The *in-vivo* analysis of captopril is difficult because of its stability concerns; the presence of sulphydrylgroup causes its self-dimerization, resulting in formation of captopril disulfide. Moreover, captopril also binds to endogenous compounds such as cysteine, glutathione as well as plasma proteins. The captopril disulfide is pharmacologically inactive but it may serves as reservoir of active drug due to its reversible conversion to free captopril.[352-354]

To overcome the above mentioned problem, the detection or measurement of free captopril concentration needs molecule derivatization or an addition of chemical stabilizer in biological samples to prevent the formation of captopril disulphide.[353] Various Fluorescence or UV active agent such as *N*-(1-pyrenyl) maleimide(NPM) and *p*-bromophenacyl bromide(*p*-BPB), have been used as chemical stabilizers. The formation of captopril disulfide can be controlled by lowering the pH below 4, adding chelating agents (EDTA) or antioxidants.[355-358] Dithiothreitol (DTT) added to the plasma samples has ability to reconstitutes captopril from its disulfide, by increasing free thiol content from serum albumin.[359, 360]

For the determination of captopril or its metabolites in blood or plasma, several analytical methods have been reported. The most widely investigated are HPLC methods, including HPLC with florescence and UV detector,[361-365] gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) techniques, fast solid phase extraction (SFE) or liquid- liquid extraction based on several evaporation and concentration steps. Other techniques like enzyme immunoassay and radioimmunoassay (RIA) have also been investigated.[366-369] The method should be successfully applied to accurately measure total captopril concentration on a large number of plasma samples.

The present work describes a simple HPLC method for the determination of captopril using UV detector. The values for LOD, precision of area and linearity show good performance of analysis. The pharmacokinetics and the relative bioavailability of captopril were studied using blood samples of 24 healthy rabbits. A written approval for required animal study was taken from Pharmacy Research Ethics Committee (PREC), The Islamia university of Bahawalpur, Pakistan.

The objective of this work was to evaluate the bioavailability of captopril from three controlled release hydrogel formulations (SP_5, R_3 and S_3). The High Performance Liquid Chromatography (HPLC) method with UV detector, using liquid-liquid extraction was developed and successfully utilized for the determination of captopril in plasma.

6.2 EXPERIMENTAL METHODS

6.2.1 Instrumentation and analytical conditions

Agilent 1100 series HPLC system consisted of LC-10 AT VP pump, DGU-14 AM on-line degasser, Rheodyne manual injector fitted with a 20 µL loop, and SPD-10 AVP UV–VIS detector and a Hypersil BDS C 8 (250 X 4.6 mm) column was utilized for separation. Chromatographic system was integrated via Shimadzu model CBM- 102 Communication Bus Module to P-IV computer loaded with CLASS-GC software (Version 5.03) for data acquisition and mathematical calculations.Centrifuge Machine (Model 4000-China), Vortex Mixer (Seouline BioScirnce-Korea), pH Meter (WTW pH 300-Germany), Ultrasonic Bath (Fisher Scientific FS 28 H-Germany), Electric Balance (Percia XB 120A), Membrane Filter (Sartorius, 0.45µm filters-Germany),Distillation Plant, Micropipettes (Softpet- Finland), Filtration Assembly (Pyrex-France), Distillation Plant (WDA/4 R & M England).

6.2.2 Materials

Captopril was a gift from Benson Pharmaceuticals, Industrial Area, Islamabad, Pakistan. All organic solvents used for the mobile phase and extraction procedure were of HPLC grade. Methanol, Acetonitrile, Orthophosphoric acid, Dichloromethane were purchased from Merck (Germany), Diethyl-ethyl ether from AnalaR (England) and Dithiothreitol from Sigma (USA). Plasma samples were obtained from healthy rabbits maintained under suitable conditions, not receiving any drug substance.

6.2.3 Preparation of the Mobile Phase

The mobile phase prepared was comprising of 0.1 M Potassium dihydrogen phosphate buffer (75%) and acetonitrile (25 %) in proportions. The pH of mobile phase was adjusted to pH 2.8 by orthophosphoric acid and filtered through filtration assembly.

6.2.4 Stock and working solutions

Stock solution of 100 µg mL^{-1} of captopril was prepared by dissolving 10 mg of drugs in100 mL volumetric flask using mobile phase as a diluent. Further dilutions were prepared in the range of 50-2000 ng/ml captopril. Solutions were prepared once and subjected to intra-day and inter-day variations of method and analyzed each time before drug analysis in biological samples, stored at 20°C. Then, 20 µL of these solutions were injected into HPLC system and chromatographed. Dithiothreitol (200 mmol/l) solution was prepared at concentrations of 30.84 g/l. It was used for the detection of captopril by reconstituting it from its disulfide dimer from plasma samples.

6.2.5 Drug-plasma solution

Standard curve was constructed to determine concentration range of captopril in rabbits. Blood samples were collected from ear vein of healthy rabbits in heparin containing centrifuge tubes and immediately centrifuged at 3500 rpm for 10 min. The supernatant obtained was stored at -20°C. After thawing, the plasma was spiked with working solutions to obtain different concentrations of captopril for construction of standard curve. All calibration curve samples (non-zero samples), except blank plasma, were prepared by spiking blank plasma aliquots of 500 µl each, with 100 µl of the intermediary captopril solutions, to yield final plasma concentrations of 50, 100, 200, 400, 800, 1200, 1600 and 2000 ng/ml.

6.2.6 Chromatographic Analytical Conditions

An Isocratic High performance Liquid Chromatography system (Agilent 1100 Series) comprising of Quanta Pump and Degasser was used. Hypersil BDS C 8 (250 X 4.6 mm) column was utilized for detection of analytes. The HPLC analysis was performed at ambient temperature, using a flow rate 1ml/min. The UV-detector was set at λ max of 205 nm.

6.2.7 Sample Extraction

Drug was extracted from plasma samples, using liquid- liquid extraction technique. To above formed samples for calibration curve, ranging from 50 to 2000 ng/ml, 3mL diethyl ether/dichloromethane (65/35) along with 0.04 mL of 200 mM dithiothreitol solution were added and the samples were vortex-mixed for 30 to 40 seconds. The tubes were then centrifuged at 3000 rpm for 5min. The upper organic layer was carefully removed, transferred to reacti-vials, and evaporated to dryness

with a gentle stream of nitrogen in a dry bath at 37ºC. A 100 µL aliquot of mobile phase, comprised of phosphate buffer (75%), acetonitrile (25%) and orthophosphoric acid (0.1%), was added to the tubes, which were then vortex-mixed for 15 seconds to reconstitute the residue. Then 20 µL were injected into liquid chromatography system and the peak areas were noted for each concentration.

6.3 Method validation

6.3.1 Specificity

Plasma samples were collected randomly from three rabbits and three humans (drug free subjects). Plasma samples from human and rabbit were taken to determine the extent to which endogenous plasma components could affect the retention time of captopril. They were spiked with captopril standard solution at three levels of drug concentrations 400 ng/ml, 800 ng/ml and 1400 ng/ml. The spiked plasma samples were processed by liquid-liquid extraction procedure and chromatographed for determination of peak area and retention time.

6.3.2 Linearity and Standard Curve Preparation

Standard curves were constructed using eleven non-zero calibration points ranging from 50 to 2000 ng/ml. The plasma samples were spiked with drug solutions to obtain final concentration of 50, 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600 and 2000 ng/ml. These samples were prepared in duplicate and subjected to liquid-liquid extraction. Five replicates of all drug concentration of each plasma sample were injected into HPLC and Chromatographed. The average of their peak area was calculated and standard calibration curves were constructed of both plasma samples (by plotting peak area versus concentrations of the samples). Linear least-square regression analysis, with weighing fact were of 1/x, was performed to assess the linearity, as well as to generate the standard calibration equation: $y = ax + b$, where y is the peak–area ratio, x the concentration, a the slope and b is intercept of regression line.

6.3.3 Lowest Limit of detection (LLOD) and quantitation (LLOQ)

The lowest limit of detection (LLOD) is a minimum amount of substance in a sample that could only be detected but not necessarily quantitated, while the quantitation limit (LLOQ) is the lowest amount of analyte in a sample which can be quantitatively determined properly with an accuracy and precision.

6.3.4 Precision and accuracy

The calibration curve samples prepared were taken for assessment of precision and accuracy. The intra-batch precision and accuracy was determined among five replicates from each batch (batch 1 and 2 batch). The average of all concentrations determined in both batches was calculated separately. Then, batch 1 and batch 2 were subjected to inter-batch precision and accuracy. Accuracy is percantage of mean of values to the reference value as given by equation:

$$\text{Accuracy} = \% \text{ mean} / \text{Reference} \qquad \text{(i)}$$

Whereas precision is percent coefficient variance (% CV) of standard deviation to mean of values:

$$\text{Precision} = \text{S.D} / \text{mean} \qquad \text{(ii)}$$

6.3.5 Extraction efficacy

Extraction efficacy (Recovery) was calculated by comparing peak-area of extracted sample of drug to that of the unextracted pure drug solutions used for plasma spiking.Three concentrations (50-2000 ng/ml) of captopril were selected to determine extraction efficiency. The peak area of spiked plasma samples were compared with standard diluents, using five replicates of each concentration.

6.4 Results and Discussions

The retention time observed by injecting 20µL standard captopril solution into HPLC system was 6.5 as shown in figure 2.

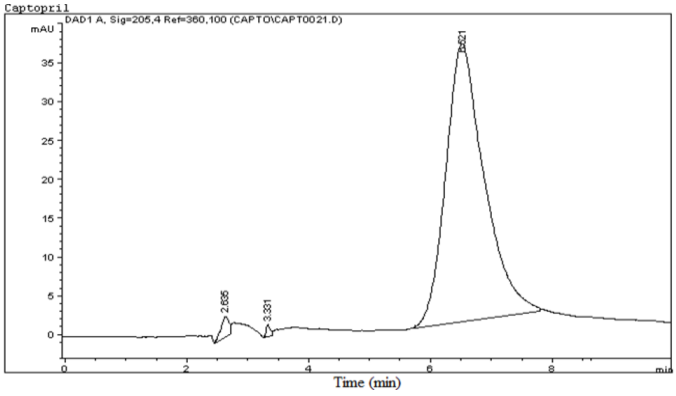

Figure 2. Retention time of captopril detected from standard drug solution

6.4.1 Specificity

The detection of captopril from rabbit and human plasma samples were compared for their respective peak area and retention time. Figure 3 and 4 represent the chromatograms of blank plasma sample of rabbit and human, respectively. There was no marked variation observed in peak area and retention time of captopril from chromatograms of spiked rabbit plasma and spiked human plasma as shown in figure 5 and 6, respectively. The retention time remained in range of 6.4 to 6.5 min in both plasma samples.

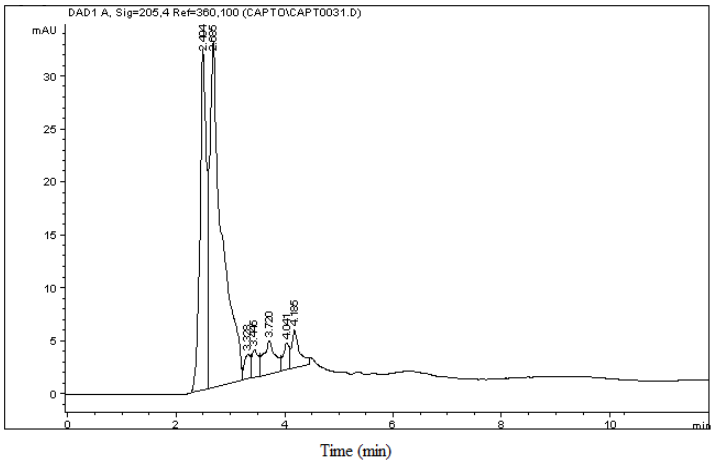

Figure 3. Blank plasma sample of rabbit

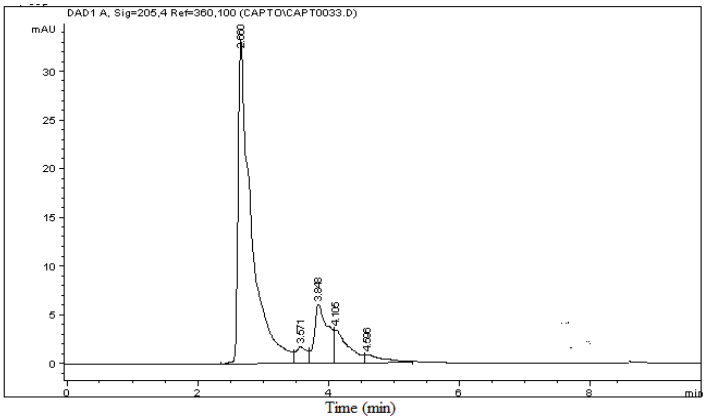

Figure 4. Blank Plasma samples of Human

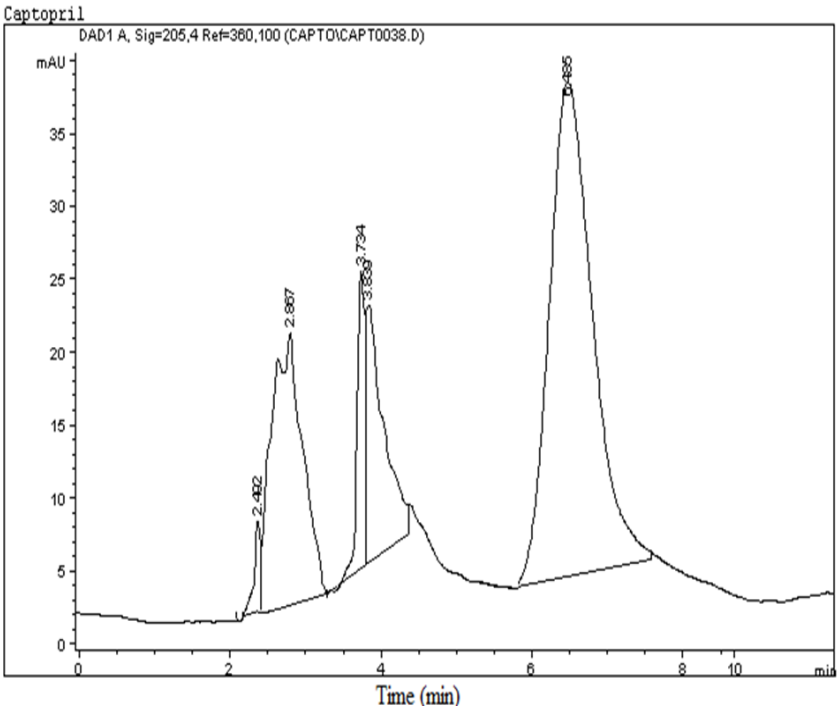

Figure 5. Captopril detected from spiked rabbit plasma sample

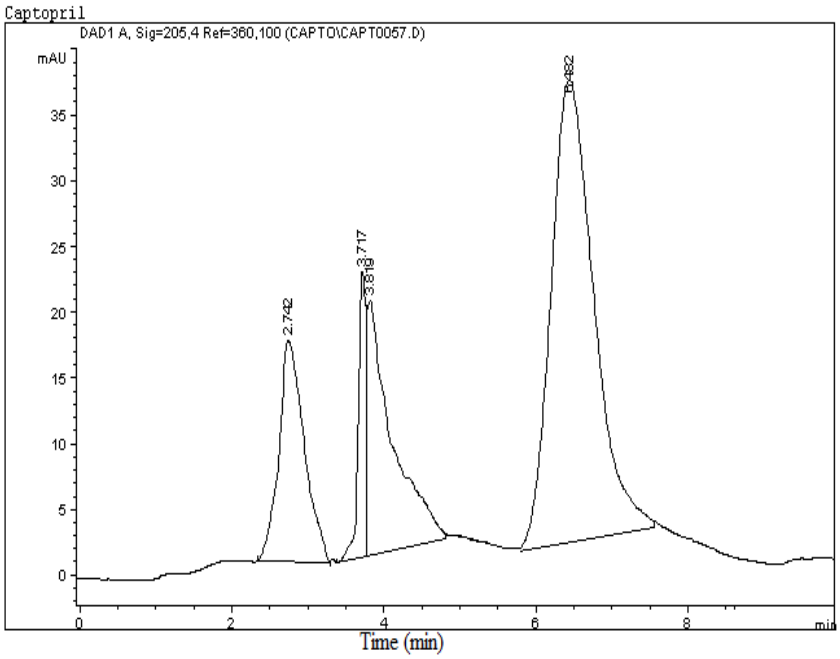

Figure 6. Captopril detected from spiked human plasma sample

The peak areas noted from spiked plasma samples from both sources (rabbit plasma and human plasma) with three levels of captopril concentrations 400ng/ml, 800ng/ml and 1400ng/ml are presented in table 1 as given below.

Table 1. Peak area determined from Rabbit Plasma and Human Plasma

QC sample (ng/ml)	Peak area (from Rabbit Plasma) (mAU*)			Average Peak area (mAU*)	Peak area (from Human Plasma) (mAU*)			Average Peak area (mAU*)
400	362.36	371.27	359.72	364.45	366.23	358.34	359.72	361.43
800	761.66	770.24	760.29	764.06	756.34	759.45	767.29	761.02
1400	1359.56	1368.26	1362.35	1363.39	1370.34	1374.31	1353.55	1366.06

It was observed from the retention time and peak area determination (as illustrated by figure 5 and figure 6) that there was no marked variation for detection of captopril from both plasma samples (rabbit and human). Hence, this method could be specifically applied for detecting captopril in plasma samples in human and animal (rabbit).

The determination of specificity of captopril was relevant to the evaluations made by Rezende *et al.*[361] in terms of using, randomly selected normal plasma, hyperlipemic and hemolyzed plasma samples from distinct healthy human subjects. However, in the current method, plasma was taken from healthy animal (rabbit) as well as human and compared, which resulted in no marked variation in retention time of captopril in the presence of endogenous plasma components from both types of plasma samples.

6.4.2 Lowest Limit of detection (LLOD) and quantification (LLOQ)

The sensitivity of this liquid-liquid extraction can be evaluated by determination of lowest limit of detection (LLOD) and lowest limit of quantification (LLOQ). By this liquid- liquid extraction method, the lowest detection limit of captopril was found as 20 ng/ml. This amount of drug in standard solutions and spiked plasma samples could be detected only, but cannot be quantified. The lower limit of detection was lower in comparison to the value of LLOD determined by Du *et al.*[370] and Alves Soares *et al.*,[360] where dithiothreitol in same quantity was added during liquid-liquid extraction. The variation in detection was because in that case mass spectrometer was used for detection, while in our method the drug was detected by UV detector. However, HPLC-UV detection is less senseitive but more simple and could be conveniently performed.

The other parameter, LLOQ that is the lower limit of quantification, was determined as 50ng/ml. Therefore, 50ng/ml was the lowest quantity of captopril that could be measured and effectively quantified. For this reason it was considered as lowest concentration in the preparation of standard curve and in plasma samples for determination of precision and accuracy.

6.4.3 Linearity and Calibration Curve

Figure 7a and 7b show the calibration curves (1 and 2) prepared from spiked plasma samples ranging from 50- 2000 ng/ml. A good linear response to this method was observed in the concentrations ranging from 50, 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600 and 2000 ng/ml as shown in the calibration curves.The drug concentrations used in preparing standard curve 1 and standard curve 2 were named as batch 1 and batch 2, respectively.

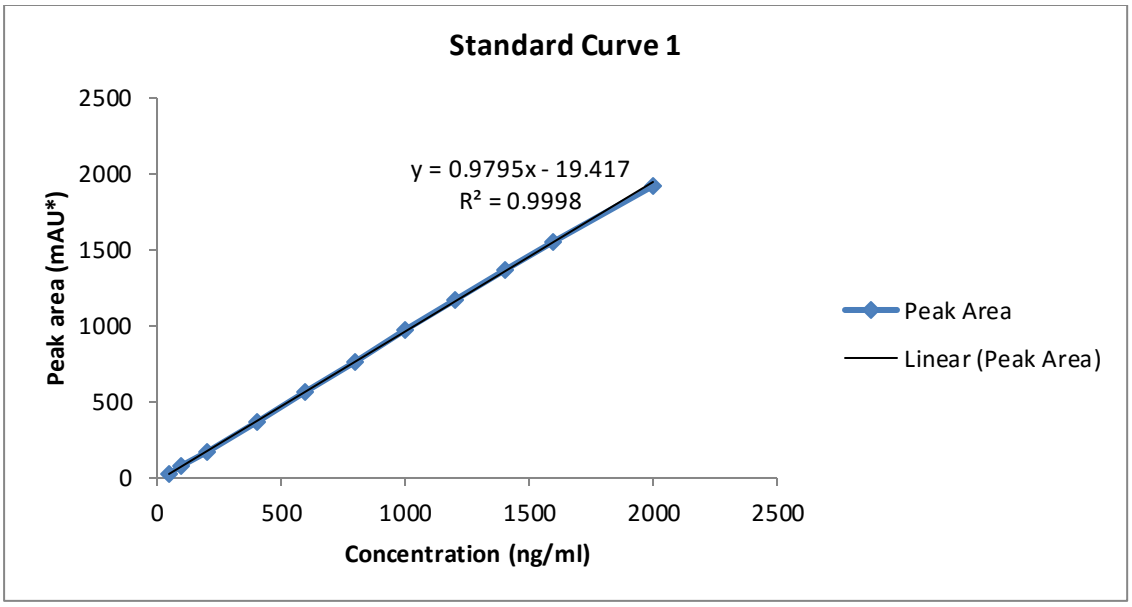

Figure 7a. Standard calibration curves prepared from spiked plasma samples of batch 1

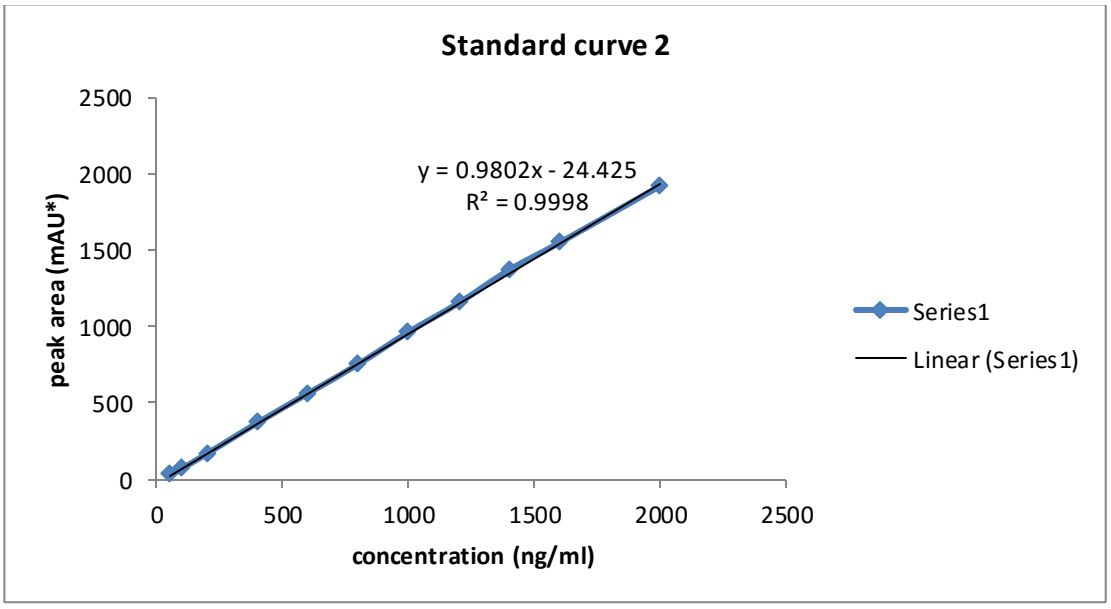

Figure 7b. Standard calibration curves prepared from spiked plasma samples of batch 2

Five replicates of each concentration were injected into HPLC and chromatographed in both batches (1 and 2). Then, mean of peak area of five replicates of each concentration in a batch was calculated and standard curves were prepared for batch 1 and batch 2. The standard curves, their slope, intercept and the correlation coefficient were determined, which could be seen in figure 7a and 7b.

6.4.4 Precision and accuracy

Intra-batch accuracy and precision evaluated are presented in table 2 (for batch 1 and for batch 2). Accuracy and precision between two batches (batch 1 and batch 2) of plasma samples used for calibration curves were determined as shown in table 3. Within run precision and accuracy of five replicates of each concentration was calculated in each batch.

Table 2. Intra-batch precision and accuracy for captopril determination in spiked plasma samples

Analysis of Batch 1									
Drug concentration (ng/ml)	Five replicates of captopril concentrations determined in rabbit plasma					mean	Standard Deviation	Precision (%CV)	Accuracy
	1	2	3	4	5				
50	49.223	51.843	46.373	46.893	48.533	48.573	2.1667	4.4608	97.146
100	88.66	94.43	93.47	90.763	98.153	93.097	3.62	3.8950	93.097
200	194.57	192.64	177.18	193.43	181.26	187.81	8.0079	4.2636	93.909
400	390.52	396.70	391.26	382.59	388.34	389.88	5.1073	1.3099	97.471
600	571.09	587.94	590.82	593.67	596.85	588.07	10.052	1.7093	98.013
800	763.83	774.18	807.09	786.09	802.05	786.65	18.245	2.3193	98.331
1000	991.29	998.64	989.48	994.66	986.18	992.05	4.7926	0.4831	99.205
1200	1174.1	1181.5	1198.0	1175.0	1189.8	1183.7	10.175	0.8595	98.644
1400	1391.8	1385.0	1395.3	1370.5	1398.0	1388.1	10.990	0.7916	99.155
1600	1563.0	1568.9	1561.0	1557.8	1570.8	1564.3	5.4272	0.3469	97.771
2000	1950.6	1946.0	1931.4	1932.3	1939.0	1939.9	8.3953	0.4327	96.996
Analysis of Batch 2									
Drug concentration (ng/ml)	Five replicates of captopril concentrations determined in rabbit plasma					mean	Standard Deviation	Precision (%CV)	Accuracy
	1	2	3	4	5				
50	43.963	48.353	50.043	46.373	47.693	47.285	2.2793	4.8203	94.5705
100	85.263	89.073	90.093	95.083	93.093	90.521	3.7866	4.1831	90.5212
200	180.07	189.02	191.53	192.30	179.36	186.45	6.2770	3.3664	93.2296
400	380.82	388.77	394.35	392.59	386.11	388.53	5.3785	1.3843	97.1328
600	589.33	587.15	578.02	586.01	591.19	586.34	5.0608	0.8631	97.7238
800	784.63	781.59	787.89	783.19	798.93	787.24	6.20025	0.787587	98.406
1000	988.46	975.27	987.62	989.25	989.86	986.02	5.463	0.554	98.609
1200	1163.2	1169.9	1173.2	1178.1	1181.1	1173.1	7.0313	0.5993	97.7634
1400	1386.0	1387.3	1391.7	1388.2	1392.4	1389.1	2.8249	0.2033	99.2266
1600	1556.0	1560.4	1569.7	1575.1	1573.4	1566.9	8.3716	0.5342	97.9360
2000	1931.8	1936.4	1944.1	1937.7	1943.4	1938.7	5.1113	0.2636	96.9368

Table 3. Inter-batch precision and accuracy for captopril determination in spiked plasma samples

Drug concentration (ng/ml)	Average of replicates in Batches (1 and 2)		Mean	Standard Deviation	Precision (%CV)	Accuracy
	Batch 1	Batch 2				
50	48.573	47.285	47.929	0.910754	1.900214	95.858
100	93.097	90.521	91.809	1.821507	1.984018	91.809
200	187.81	186.45	187.13	0.961665	0.513902	93.565
400	389.88	388.53	389.205	0.954594	0.245268	97.30125
600	588.07	579.94	584.005	5.748778	0.984371	97.33417
800	786.65	771.44	779.045	10.75509	1.380549	97.38063
1000	992.05	982.09	987.07	7.042784	0.713504	98.707
1200	1183.7	1173.1	1178.4	7.495332	0.63606	98.2
1400	1388.1	1389.1	1388.6	0.707107	0.050922	99.18571
1600	1564.3	1566.9	1565.6	1.838478	0.11743	97.85
2000	1939.9	1938.7	1939.3	0.848528	0.043754	96.965

In batch 1, the values of precision are ranging from 0.34% to 4.4% and and accuracy determined is in the range of 93.00% to 99.15%. Similarly, the values of precision and accuracy in batch 2 were ranging from 0.26% to 4.8% and 90.53% to 99.22%, respectively.

The inter-batch precision and accuracy was in the range of 0.04 to 1.98 and 90.8 to 99.18, respectively. The results obtained were within the acceptance criteria for precision and accuracy. It was assessed from deviation values that were falling in ±15% of the authentic values.[371] The determination of intra- batch and inter-batch precision and accuracy were according to that determined Rezende *et al.,*[361] where intra-batch precision and accuracy (% CV) ranged from 2.49 to 5.66%, and 97.15 to 105.77%, respectively. Method inter-batch precision (% CV) and accuracy ranged from 0.88 to 1.95%, and 98.85 to 104.22%, respectively. Similar findings were observed by Alves Soares *et al.,*[360] where liquid-liquid extraction was used for detection of captopril.

Moreover, the retention time of noted in all chromatograms of different concentrations and within batch and among batches was ranging from 6.4 to 6.7 which was near to the retention time of captopril determined by Jinsong *et al.*[372]

6.4.5 Extraction efficacy

Extraction efficacy (Recovery) was calculated by comparing the peak-area of extracted sample of drug to that of the unextracted pure drug solutions used for plasma spiking as presented in table 4. The pure Captopril solutions of concentrations (50, 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600 and 2000 ng/ml) were considered as standard for comparison with spiked plasma samples. The peak area of spiked plasma samples were compared with standard diluents, using five replicates of each concentration (50, 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600 and 2000 ng/ml). Their averages were calculated and then comparison was made to determine the percentage of drug that could be possibly recovered from spiked plasma samples. The percent recoveries of all samples with drug concentrations ranging from 50 to 2000 ng/ml are presented in table 4.

Table 4. Percent Recoveries of different Captopril concentrations from spiked plasma

Drug concentration (ng/ml)	Peak area of spiked plasma (mAU*)	Peak area of standard Solution (mAU*)	% Recovery
50	28.76	29.62	97.09
100	73.28	77.37	94.71
200	168.01	176.43	95.22
400	370.07	374.29	98.87
600	568.26	572.39	99.27
800	766.84	767.13	99.96
1000	972.24	978.81	99.33
1200	1163.93	1169.42	99.53
1400	1368.36	1378.29	99.27
1600	1544.53	1576.45	97.97
2000	1920.13	1954.78	98.23

The values of percent recovery are varying from approximately 95.00% to 99.96%. The mean percent recovery of captopril from the spiked plasma samples was 98.13%. These results indicated a successful recovery of drug from biological samples, as more than 95% of drug could be detected from plasma samples. Therefore, it evaluates the suitability of method for effective determination of captopril from blood after its oral administration. The results of percent recovery were determined in accordance to extraction efficiency (% recovery) measured by Rezende *et al.*[361] and Sultan *et al.*[273] where percent recoveries of captopril were calculated in the similar manner.

6.5 Application of the Method

The objective of this work was to evaluate the bioavailability of captopril from three controlled release hydrogel formulations. The High Performance Liquid Chromatography (HPLC) method with UV detector, using liquid- liquid extraction was developed and utilized for the determination of captopril in plasma of rabbits.

Twenty four (24) rabbits weighing 2.0 to 2.5kg were used in this study. They were divided into four groups (1, 2, 3 and 4) each group having six rabbits. Animals were starved for 24h prior to administration of the drug. Each rabbit in group 1 (Control) was given a single dose of 25 mg pure captopril with lactose enclosed in a hard gelatin capsule, which was ingested with 20 mL water orally through a catheter. Rabbits in group 2, 3 and 4 were given hydrogel formulations SP_5, R_3 and S_3, respectively. Water was provided during starvation and throughout the experiment. Food was withheld for 2 hours following drug administration to prevent drug food interaction, after which they were fed at 4h and 10h after dosing.

Blood samples (3 mL each) were collected into heparin-containing centrifuge tubes at 0, 0.5, 1, 2, 4, 8, 12,16, 24 hours from the rabbit ear vein following drug administration. Plasma was separated witha centrifuge (Heraeus Instrument, Biofuge Primo, Germany) at 3500 rpm for 10 min at room temperature and the plasma recanted and stored at –20ºC until assayed for captopril content. Plasma captopril concentrations were determined in duplicate by reversed phase liquid chromatography-UV.

Briefly, 3mL diethyl ether/dichloromethane (65/35) was added to 0.5mL plasma with 0.04mL of 200mM dithiothreitol soltion, and the samples were vortex-mixed for 30 to 40 seconds. The tubes were then centrifuged at 3500rpm for 5min at 4ºC. The upper organic layer was carefully removed, transferred to reacti- vials, and evaporated to dryness with a gentle stream of nitrogen in a dry bath at 37ºC. A 100 µL aliquot of mobile phase, comprised of phosphate buffer (75%), acetonitrile (25%) and orthro phosphoric acid (0.1%), was added to the tubes, which were then vortex-mixed for 15 seconds to reconstitute the residue. Then 20 µL were injected into liquid chromatography system.

The drug was eluted with phosphate buffer and acetonitrile (75:25) using orthophosphoric acid to adjust the pH to 3. It was filtered by a vacuum filter system equipped with a 0.8 mm filter and was degassed by ultrasonic treatment.

6.5.1 Operating conditions

The flow rate was 1.0 mL/min at ambient temperature and UV detection was performed at 205 nm. The total run time was 10 min and retention time was 6.5 min.

6.5.2 Plasma Concentrations Profile and Pharmacokinetic parameters of Captopril

Plasma concentrations of administered pure captopril and drug released from hydrogels were calculated from peak area by using Microsoft excel 2010.

The pharmacokinetic parameter such as maximum concentration C_{max} (ng/ml), time to reach maximum concentration, T_{max} (hr), elimination half-life '$t'_{1/2\ el}$, elinmination rate constant k_e (hr^{-1}) Area under curve AUC (ng/ml*hr), area under the product of concentration and time AUMC (ng /ml*h^2), Clearance (L/h) , volume of steady state concentration V_{ss} (L) volume of distribution V_d (L), and other parameters mentioned in tables 4 to 27 were calculated using Kinetica 5.0.

The value of absorption half-life $t_{1/2\ (a)}$ was calculated by method of inspection. From the value of absorption half-life $t_{1/2\ (a)}$, the absorption rate constant k_a was estimated. Following equations were used for the calculation of absorption half-life $t_{1/2\ (a)}$ and absorption rate constant k_a.

$$t_{1/2\ (a)} = \frac{t_{max}}{5} \qquad (1)$$

$$k_a = \frac{0.693}{t_{1/2}} \qquad (2)$$

6.5.2.1 GROUP 1

The mean ± standard deviation concentrations of captopril determined in plasma of six rabbits (group 1) after oral administration of control are presented in table 5. Figure 8 illustrates mean ± standard deviation of plasma concentrations versus time profile of Captopril. The values of mean ± standard deviation of Bioavailability and Pharmacokinetic parameters are given in table 6.

Table 5. Mean ± standard deviation of plasma concentration (ng/ml) of Captopril (free drug) in 6 rabbits in Group 1

S. No.	Time (Hrs)	Concentration (ng/ml) Captopril
1	0	0 ± 0
2	0.5	442.31 ± 21.70
3	1	782.18 ± 12.69
4	2	639.31 ± 21.19
5	4	363.55 ± 47.93
6	8	52.288 ± 5.35
7	12	0 ± 0
8	16	0 ± 0
9	24	0 ± 0

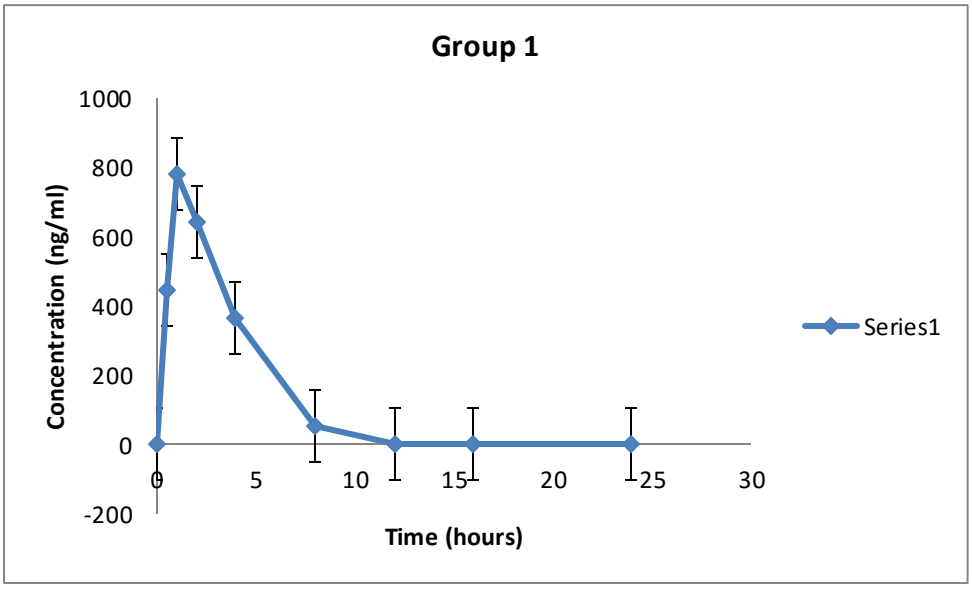

Figure 8. Mean ± standard deviation of plasma concentrations vs. time profile of Captopril 25 mg plotted on rectangular co-ordinate graph, in group 1.

Table 6. Mean ± standard deviation of Bioavailability and Pharmacokinetic parameters of Captopril administered in an oral dose of 25 mg (free drug) in rabbits of Group 1.

Parameters	**Captopril (free drug)**
C_{max} (ng/ml)	782.198 ± 12.68
T_{max} (hr)	1 ± 0
AUC (ng /ml*h)	3092.25 ± 176.03
AUMC (ng /ml*h^2)	9144.63 ± 682.72
MRT (hr)	2.96 ± 0.081
k_e (hr^{-1})	0.425 ± 0.017
k_a (hr^{-1})	3.46 ± 0
Tlast (hr)	2.63 ± 0.0613
$t_{1/2\ el}$ (hr)	1.61 ± 0.066
$t_{1/2\ (a)}$(hr)	0.2 ± 0
V_{ss} (L)	23.94 ± 1.047
V_d (L)	18.98 ± 1.38
Cl_T (L/h)	8.106 ± 0.48
R^2	0.987 ± 0.012
HVD (hr)	3.32 ± 0.317

From the results presented in table 5 and table 6, it can be concluded that maximum level of drug concentration was attained in a sample taken after 1 hour of oral administration of captopril powder enclosed in hard gelatin capsule, hence time to reach maximum concentration t_{max} was 1 hour. Because of solubility of captopril in aqueous solution and administration in powder form, it was immediately dissolved in biological fluids and reached its maximum concentration in blood in shorter time. It was detected from the plasma samples of rabbits taken upto a time period of 8 hours.

Similar results were also evaluated by Mallick *et al.*[374] in assessment of bioavailability and pharmacokinetic parameters of another drug nifedipine from controlled release micro capsules in healthy rabbits. In their research work the control (reference) used was drug enclosed in hard gelatin capsules and orally administered in a group of rabbits, as it was used in our work for captopril.

6.5.2.2 GROUP 2

The mean ± standard deviation concentrations of captopril determined in plasma of six rabbits (group 2) after oral administration of SP5 hydrogel formulation are presented in table 7. Figure 9 illustrates mean ± standard deviation of plasma concentrations versus time profile of Captopril. The values of mean ± standard deviation of Bioavailability and Pharmacokinetic parameters are given in table 8.

Table 7. Mean ± standard deviation of plasma concentration (ng/ml) of Captopril released from SP5 hydrogel formulation in 6 rabbits in Group 2

S. No.	Time (Hrs)	Concentration (ng/ml) Captopril
1	0	0 ± 0
2	0.5	151.49 ± 8.48
3	1	361.19 ± 17.37
4	2	485.08 ± 23.36
5	4	661.85 ± 5.78
6	8	611.88 ± 11.58
7	12	378.87 ± 12.19
8	16	186.31 ± 22.92
9	24	50.570 ± 5.29

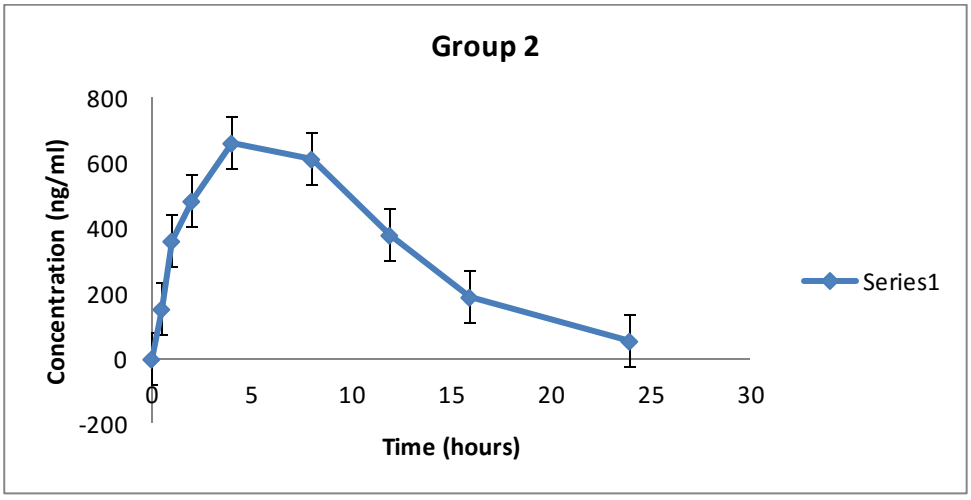

Figure 9. Mean ± standard deviation of plasma concentrations vs. time profile of Captopril released from SP5 hydrogel formulation plotted on rectangular co-ordinate graph, in group 2.

Table 8. Mean ± standard deviation of Bioavailability and Pharmacokinetic parameters of Captopril released from SP5 hydrogel formulation in Group 2

Parameters	**Captopril (SP5 formulation)**
C_{max} (ng/ml)	661.853 ± 5.79
T_{max} (hr)	4 ± 0
AUC (ng /ml*h)	8647.81 ± 133.05
AUMC (ng /ml*h^2)	79382.36 ± 2619.9
MRT (hr)	9.17 ± 0.195
K_e (hr^{-1})	0.165 ± 0.009
k_a (hr^{-1})	0.86 ± 0
Tlast (hr)	8.41 ± 0.127
$t_{1/2\ el}$ (hr)	4.19 ± 0.28
$t_{1/2\ (a)}$(hr)	0.8 ± 0
V_{ss} (L)	26.53 ± 0.46
V_d (L)	17.5 ± 1.13
Cl_T (L/h)	2.87 ± 0.058
R^2	0.999 ± 0.0064
HVD (hr)	11.82 ± 0.146

It was shown from the data presented in table 7 and 8 that the maximum concentration of drug was achieved in 4 hours after oral administration of SP5 hydrogel formulations. The average C_{max} was 661.85ng/ml, which was lesser than average C_{max} (782.19ng/ml) noted after oral administration of control. However, time to reach maximum concentration was 4 hours that was higher in comparison control. Area under curve (AUC) and area under the product of concentration and time (AUMC) were also increased.

The results indicated from the estimation of pharmacokinetic and bioavailability parameters that the drug was released from the gastroretentive hydrogel formulation SP5 at controlled rate and maintained its concentration in blood for longer periods of time. The drug released by gastroretentive hydrogel was in correspondence with that observed by Nagarwal *et al.*,[375] for determination of release of Cinnarizine Hydrochloride in rabbits blood from its Gastroretentive Tablet. The pharmacokinetic parameters and bioavailability of Cinnarizine Hydrochloride Gastroretentive Tablet were compared with reference (oral suspension) in healthy rabbits in the similar manner.

6.5.2.3 GROUP 3

The mean ± standard deviation concentrations of captopril determined in plasma of six rabbits (group 3) after oral administration of R3 hydrogel formulation are presented in table 9. Figure 10 illustrates mean ± standard deviation of plasma concentrations versus time profile of Captopril. The mean ± standard deviation of Bioavailability and Pharmacokinetic parameters are given in table 10.

Table 9. Mean ± standard deviation of plasma concentration (ng/ml) of Captopril released from R3 hydrogel formulation in 6 rabbits in Group 3

S. No.	Time (Hrs)	Concentration (ng/ml) Captopril
1	0	0 ± 0
2	0.5	110.5 ± 11.99
3	1	221.78 ± 31.94
4	2	381.43 ± 17.1
5	4	491.09 ± 39.8
6	8	598.48 ± 15.33
7	12	512.11 ± 17.63
8	16	329.25 ± 13.68
9	24	61.15 ± 11.44

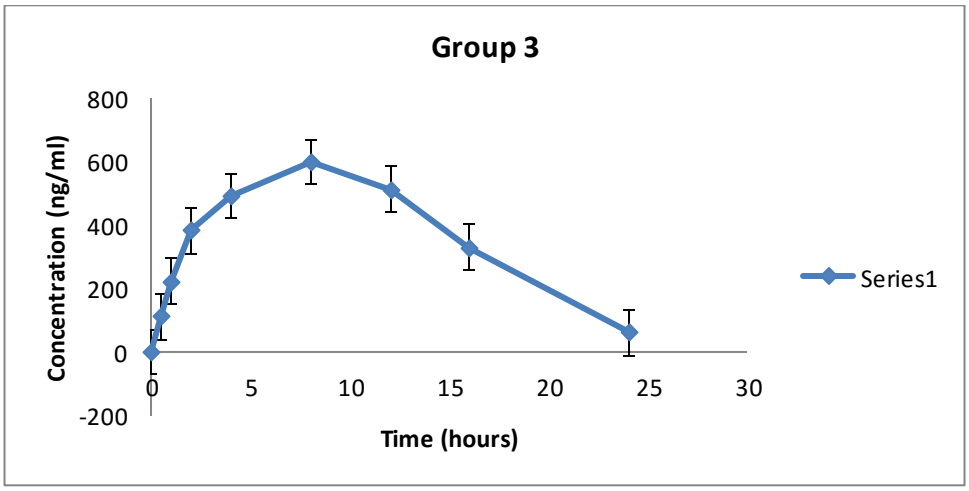

Figure 10. Mean ± standard deviation of plasma concentrations vs time profile of Captopril released from R3 hydrogel plotted on rectangular co-ordinate graph, in group 3

Table 10. Mean ± standard deviation of Bioavailability and Pharmacokinetic parameters of Captopril Captopril released from R3 hydrogel formulation in 6 rabbits in Group 3

Parameters	**Captopril (R3 formulation)**
C_{max} (ng/ml)	598.48 ± 15.33
T_{max} (hrs)	8 ± 0
AUC (ng /ml*h)	9320.255 ± 222.97
AUMC (ng /ml*h^2)	11295.45 ± 964.66
MRT (hr)	10.88 ± 0.175
k_e (hr^{-1})	0.173 ± 0.006
k_a (hr^{-1})	0.43 ± 0
Tlast (hr)	10.03 ± 0.109
$t_{1/2\ el}$ (hr)	3.95 ± 0.16
$t_{1/2\ (a)}$(hr)	1.6 ± 0
V_{ss} (L)	29.21 ± 0.99
V_d (L)	15.31 ± 0.704
Cl_T (L/h)	2.678 ± 0.067
R^2	0.9774 ± 0.013
HVD (hr)	14.94 ± 0.47

The results presented in table 8 and table 10 as well as depicted from figure 10, that time to reach maximum concentration T_{max} of captopril after oral administration captopril loaded R3 hydrogel was 8 hour. It was higher than one observed after administering captopril loaded SP5 hydrogel in group 2. The maximum concentration C_{max} attained (598.48 ng/ml) was reduced in comparison to control. The values of mean AUC and mean AUMC were higher even when compared to that in group 2. The captopril was released from R3 formulation and detected in plasma samples upto 24 hours. It could be evaluated from the data obtained in group 3, that captopril was released at slower rate from the controlled release hyfrogel formulation R3 and remained available in blood for longer duration.

6.5.2.4 GROUP 4

The mean ± standard deviation concentrations of captopril determined in plasma of six rabbits (group 4) after oral administration of S3 hydrogel formulation are presented in table 11. Figure 10 illustrates mean ± standard deviation of plasma concentrations versus time profile of Captopril. The values of mean ± standard deviation of Bioavailability and Pharmacokinetic parameters are given in table 12.

Table 11. Mean ± standard deviation of plasma concentration (ng/ml) of Captopril released from S3 hydrogel formulation in 6 rabbits in Group 4

S. No.	Time (Hrs)	Concentration (ng/ml) Captopril
1	0	0 ± 0
2	0.5	131.4134 ± 29.31
3	1	277.95 ± 22.08
4	2	408.065 ± 14.02
5	4	557.54 ± 11.92
6	8	618.8584 ± 8.89
7	12	512.0184 ± 30.56
8	16	215.585 ± 29.69
9	24	0 ± 0

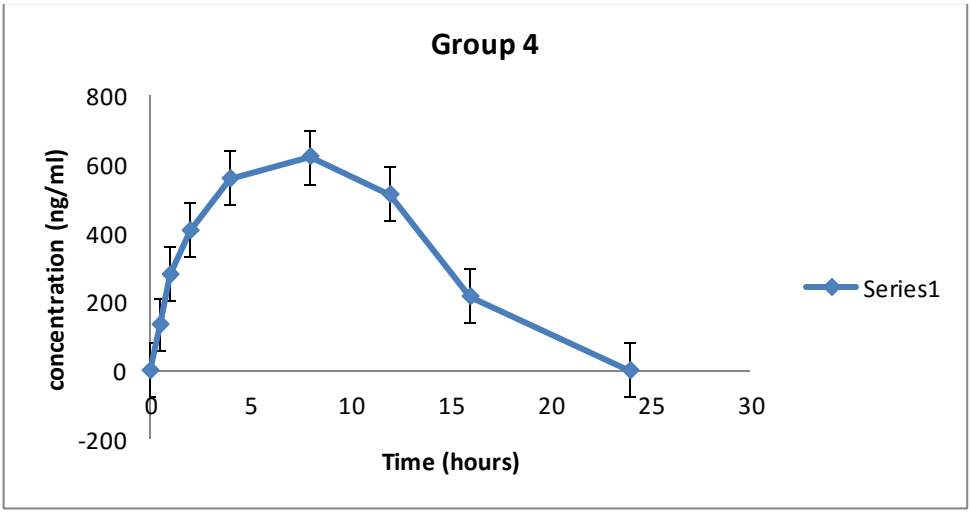

Figure 11. Mean ± standard deviation of plasma concentrations vs. time profile of Captopril released from R3 hydrogel formulation plotted on rectangular co-ordinate graph in group 4.

Table 12. Mean ± standard deviation of Bioavailability and Pharmacokinetic parameters of Captopril released from S3 hydrogel formulation in 6 rabbits in Group 4

Parameters	**Captopril (S3 formulation)**
C_{max} (ng/ml)	618.85 ± 8.896
T_{max} (hr)	8 ± 0
AUC (ng /ml*h)	8878.77 ± 149.743
AUMC (ng /ml*h^2)	91632.7 ± 2318.5
MRT (hr)	10.32 ± 0.188
K_e (hr^{-1})	0.145 ± 0.004
k_a (hr^{-1})	0.43 ± 0
Tlast (hr)	7.83 ± 0.045
$t_{1/2\ el}$ (hr)	4.707 ± 0.15
$t_{1/2\ (a)}$(hr)	1.6 ± 0
V_{ss} (L)	29.057 ± 0.64
V_d (L)	19.13 ± 0.6
Cl_T (L/h)	2.82 ± 0.053
R^2	0.876 ± 0.022
HVD (hr)	12.76 ± 0.18

The rabbits in group 4 were given captopril loaded S3 hydrogel formulation orally. The drug was released at slower rate and attained maximum concentration in 8 hours similar to that in group 3, hence T_{max} was 8 hour as shown in table 12. The mean of maximum concentration of captopril attained was 618.85 ng/ml. The drug remained detectable for duration of 16 hour from rabbit blood.

The results presented from *in vivo* studies on rabbits had shown a difference in plasma concentration of captopril attained after oral administration of free drug (control) and three hydrogel formulations (SP5, R3 and S3) as shown in the figure 12. A clear variation in maximum concentration of captopril achieved by control and controlled release hydrogel formulations could be seen from the figure. The drug reached its maximum concentration in short period of time in case of free drug administered in capsules. In comparison to control, a lesser as well as slower drug release was observed from drug loaded hydrogels that was dependent upon the swelling characteristics of crosslinked polymeric networks. Drug release

pattern from these hydrogels was also varying from one another due to their different constitution.

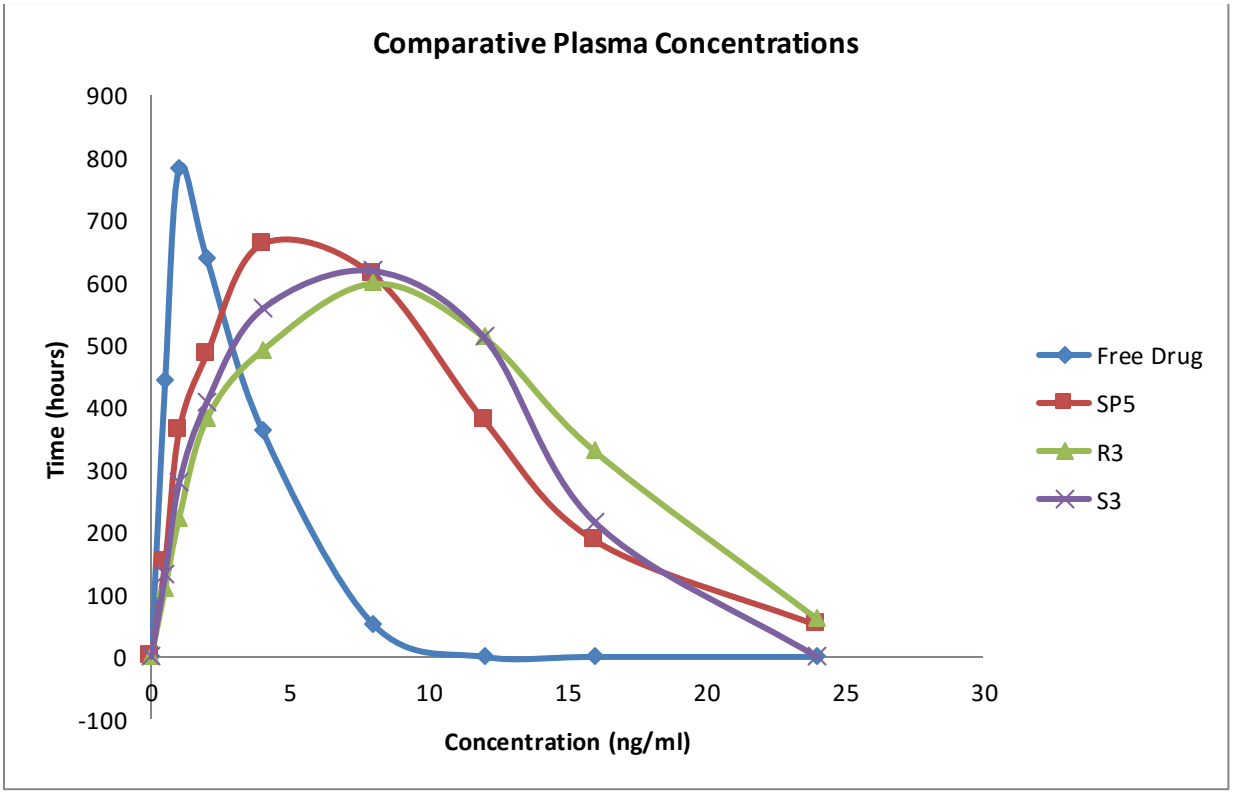

Figure 12. Comparison of Mean Plasma concentrations vs. time profile of control (free drug), Captopril released from SP5, R3 and S3 hydrogel formulation plotted on rectangular co-ordinate graph.

Therefore, the maximum concentration C_{max} was reduced while T_{max} was increased by hydrogels. The value of area under curve AUC (calculated by trapezoidal rule) and elimination half-life were higher for controlled release hydrogel formulation. The drug could be available for longer periods of time after administration of Captopril loaded hydrogels. Among the hydrogel formulations T_{max} was 4 hour for SP5 and 8 hr for R3 and S3 formulations, drug was available for 24 hours from SP5 and R3 but in S3 formulation it was detectable for relatively shorter periods (16 hours). The formulation SP5 was a super porous hydrogel and had gastro-retentive ability due to excessive swelling at acidic pH. For this reason the time to reach maximum concentration levels (4 hours) was lesser than other two formulations (S3 and R3) having maximum concentration noted at 8 hour sample. Hydrogels S and R resealed maximum drug concentrations at intestinal pH, hence

attaining maximum concentration of captopril in 8 hours. All the results obtained were subjected to statistical analysis in terms of mean ± standard deviation as presented in the tables 5 to 12.

Similar patterns of captopril release in rabbits were observed by El-Shabouri *et al.*[376] where release of captopril was evaluated from prepared captopril buccoadhesive tablets, which were compared with an immediate release captopril tablet as control. A decrement in maximum concentration and increment in time to reach maximum drug concentration was observed in the controlled release formulations. In the same manner release patterns of other drugs from their controlled release formulation have been compared with controls using HPLC analysis. For example, prepared sustained release leflunomide microcapsules were compared with conventional tablets of leflunomide (control), which had shown similar comparative drug release patterns from sustained release formulations.[377] In another work, release of theophylline from pH-Sensitive hydrogels of Carboxymethyl Chitosan had been evaluated in comparison to a commercially available theophylline tablet as control.[378] Similarly, an evaluation of the pharmacological activity of glipizide was conducted by Abdelbary *et al.*[379] on controlled release microcapsules and compared with commercially available immediate release Minidiad® 5 mg in normal healthy albino rabbits. Therefore, the controlled release drug delivery systems have ability to maintain optimum levels of drug for longer durations of time.

CONCLUSION

In short, it was concluded that the above HPLC method is specific, sensitive, rapid and easy to perform determination of captopril. The limit of quantification, small sample volume and chromatographic time of this method makes it advantageous for adaptation to routine assay requirements and enables simultaneous determination of captopril because of good separation of the chromatographic peaks of captopril from plasma peaks. Results were accurate and precise and confirmed by the statistical parameters. Reliability, rapidness, simplicity, sensitivity, economical nature, good recovery and precision of this method give it advantage over the other reported methods. Moreover, the developed method was successfully applied in determination of captopril in plasma samples following the oral administration of captopril loaded hydrogels.

Overall Conclusion

From the whole research project, including methodologies, techniques, the results obtained and entire discussion, it has been revealed that hydrogel formulations were successfully developed for controlled release drug delivery of captopril. Swellable polymeric networks with varying characteristics were prepared, by free radical polymerization, using different ratios of polymers, monomers as well as techniques. A fast technique for hydrogel synthesis was utilized by the assistance of microwave radiations, presenting better results in comparison to conventional method (using thermostatic water bath). The prepared HPMC/PVA-co-poly(acrylic acid) hydrogels had remarkable ability to entrap as well as protect drug and release it at a control rate, hence providing an opportunity for drug to exert its required therapeutic effects for prolonged durations. The other hydrogel formulations prepared, comprised of HPMC, acrylic acid along with AMPS had better swelling capability as compared to HPMC/PVA-co-poly(acrylic acid) hydrogels, due to the presence of highly ionize-able sulfonic groups.

Moreover, the gastro-retentive hydrogel formulations were prepared, which had additional advantages of significantly high swelling power and ability to be retained in stomach and releasing captopril for longer periods of time at low pH of stomach. Thus, captopril remains more stable at acidic pH and relatively lesser quantity will be able to produce enhanced therapeutic effect. These gastro-retentive polymeric networks had an optimum strength and flexibility to maintain their shape and texture during swelling measurement, drug loading and *in-vitro* drug release study, without any noticeable/apparent breaking. Other *in-vitro* characterization measurements were presenting crosslinking among polymeric networks of polymers and monomers as analyzed by FT-IR, porous structure was observed in morphological analysis by scanning electron microscopy (SEM). The X-Ray Diffraction had shown amorphous distribution or dispersion of crystalline drug in formed cross-linked structures. The thermal analysis (TGA and DSC) evaluated the desirable stability of cross-linked polymeric networks as well as stability of drug loaded into loaded into these hydrogels. The swelling properties were significantly affected by the varying ratios of monomers, polymers and cross linker in each type of hydrogel formulation. Radiation dose was an important factor affecting the swelling ratios of hydrogels synthesized under their influence, due to their ability to initiate crosslinking interactions. The drug loading and release from hydrogels were directly related to their water absorption characteristics.

HPLC-UV analytical method, using liquid-liquid extraction procedure was developed for determination of bioavailability and pharmacokinetic parameters of captopril in plasma samples of rabbits. The liquid chromatography method, due to its specificity and sensitivity had ability to detect even smaller concentrations of captopril. It was concluded from the *in-vivo* evaluation, that captopril was released from hydrogel formulations at controlled rates and lesser amounts in comparison to control. However, the drug remained available in blood for longer durations of time hence providing a safe and efficacious amount for useful effects.

Therefore, the aim of preparing a hydrogel formulation was achieved fulfilling the criteria of controlled release drug delivery system successfully by increasing time to reach maximum concentration and overall bioavailability of drug. Thus, optimum levels of drug could be maintained in blood for required therapeutic effects in the treatment of hypertension as well as other heart disorders, reducing the dosing frequency, lower incidence of side effects and ultimately improving patient compliance.